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VARIATION IN NEEDLE RETENTION OF SCOTS PINE IN RELATION TO LEAF MORPHOLOGY, NITROGEN CONSERVATION AND TREE AGE

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CONTENTS

ORIGINAL PAPERS

The thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I **Pensa M,** Jalkanen R (1999) Needle chronologies on *Pinus sylvestris* in Northern Estonia and Southern Finland. SILVA FENNICA 33: 171–177

II Jalkanen R, **Pensa M** (2000) Influence of the alkaline emissions produced by the oil-shale industry on needle retention and needle age of Scots pine. FORESTRY STUDIES (ESTONIAN AGRIC. UNIV.) 34: 79–84

III **Pensa M,** Jalkanen R, Sellin A (2001) Age-dependent changes in needle-fascicle dynamics of *Pinus sylvestris* (L.). SCANDINAVIAN JOURNAL OF FOREST RESEARCH 16: 379–384

IV **Pensa M,** Sellin A (2002) Needle longevity of Scots pine in relation to foliar nitrogen content, specific leaf area, and shoot growth in different forest types. CANADIAN JOURNAL OF FOREST RESEARCH 32: 1225–1231

V **Pensa M,** Sellin A (2003) Soil type affects nitrogen conservation in foliage of small *Pinus sylvestris* L. trees. PLANT AND SOIL 00: 1–9

VI **Pensa M,** Aalto T, Jalkanen R (2003) Variation in needle traces diameter in respect of needle morphology in five conifer species. Manuscript submitted for publication in TREES

1. INTRODUCTION

There are a variety of factors causing variation in leaf life-span of plants, of which the most important are genetically determined differences between the species and the effects of environmental conditions. Increase in leaf longevity among species has frequently been explained as an adaptation to low nutrient availability (Monk 1966, Chapin 1980, Coley *et al.* 1985) or to general conditions in which growth is suppressed by any environmental stress factor (Grime 1977, Chabot and Hicks 1982, Reich *et al.* 1992).

Intra-specific changes in the leaf longevity of evergreen conifers have commonly been observed along latitudinal or altitudinal gradients (Weidman 1939, Ewers and Schmid 1981, Schoettle 1990, Nebel and Matile 1992, Jalkanen *et al.* 1995, Xiao 2003). For example, the needle retention in *Picea mariana* (Mill.) BSP has been reported to vary from 5–7 years in southerly reaches of the boreal forest in Quebec to 13 years in central Alaska, and up to 30 years under subarctic conditions (Lamhamedi and Bernier 1994). The intra-specific differences in leaf longevity among conifer populations at different latitudes and elevations appear rather to be phenotypic acclimation than genotypic adaptation (Reich *et al.* 1996a), but the nature of the factors to which conifers have acclimated is still not unequivocally clear. Nevertheless, Weidman (1939), Jalkanen *et al.* (1995) and Xiao (2003) have reported that the variation in needle longevity of pine species was more closely related to variation in temperature regime than to site elevation or latitude *per se*.

Besides the variation across climatic zones (along altitudinal and longitudinal gradients), leaf longevity may also vary within a climatic zone if individuals of the same species in adjacent habitats are subjected to different levels of resources. The longevity of leaves in *Rhododendron maximum* L. (Nilsen *et al.* 1987) and *Acer mono* Maxim. (Seiwa 1999a) increased as the light availability decreased. In *Pinus tabulaeformis* Carr., needle longevity was greater for trees growing in shade than in sun (Xiao 2003). Field experiments on two evergreen ericaceous shrub species indicated that leaf longevity might decrease in response to fertilisation (Aerts 1989). Fertilisation of *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco and *Abies grandis* Lindl. reduced needle longevity by 26 and 27%, respectively (Balster and Marshall 2000). Similar intra-specific trends in leaf longevity have been revealed along natural gradients of nutrient and water availability (Jonasson 1989, Nordell and Karlsson 1995, Enoki and Kawaguchi 1999, Kloeppel *et al.* 2000). It has been supposed that such variation reflects the response of the trees to low resource availability or high abiotic stress level, and that leaf life-span co-varies with trees' growth rate and photosynthetic capacity (Reich *et al.* 1992, 1999). In less favourable environments it might be a successful strategy to increase the time that nutrients are resident in plant tissues, and this can be achieved in part by increasing the life-span of the foliage (Eckstein *et al.* 1999).

Besides extending the life-span of plant organs, nutrients can be conserved by increasing the efficiency of resorption. Many plant ecologists have considered the resorption of nutrients from senescent parts of plants to be a primary nutrient conservation mechanism, which enables the reuse of nutrients once accumulated in plant tissues (Aerts 1996, Eckstein *et al.* 1999, Chabot and Hicks 1982, Chapin 1980). Among 14 subarctic species studied by Eckstein and Karlsson (1997), the woody evergreens demonstrated the longest mean residence time (**MRT**) and the lowest nutrient productivity. These two properties are often accompanied by a suite of traits called 'stress resistance syndrome', which is treated as an adaptation to stressful conditions (Chapin *et al.* 1993). Studies comparing leaf life-span, nutrient use, and photosynthetic capacity across various biomes or ecosystems have shown that these characteristics are strongly related to each other and the relationships are valid within a wide range of habitats (Reich *et al.* 1992, 1999).

However, it is not evident that adjusting leaf longevity to stress factors under the same climatic conditions should be associated with a decrease in productivity and growth rate (Eckstein and Karlsson 2001), although within-species comparisons across climatic zones have shown such inverse relationships (Jalkanen *et al.* 1995, Kikuzawa 1991, Lamhamedi and Bernier 1994, Weidman 1939). Whether changes in nutrient conservation accompany this small-scale within-species variation in leaf life-span is rather unclear. In about 60% of the intra-specific experiments, nutrient-resorption efficiency, defined as a percentage of nutrients relocated from senescent tissues, did not respond to increased nutrient availability (Aerts 1996). Nordell and Karlsson (1995) presented data indicating that habitats affected nitrogen resorption in *Betula pubescens* Ehrh., but it remained unclear whether this variation was related to habitat fertility. In *Pinus radiata* D. Don, the resorption efficiency of nitrogen was not connected with soil nutrient availability (Nambiar and Fife 1987). An experiment carried out on *Betula neoalaskana* Sarg. also showed that the nutrient status of the trees did not affect the nitrogen-resorption process (Chapin and Moilanen 1991). However, there are a number of published studies which report that nutrient resorption within a species decreases in response to enhanced soil fertility; about 30–35% of the studies analysed by Aerts (1996) gave such results (see also Kost and Boerner 1985). Thus, within a species it is still unknown to what degree nutrient conservation (e.g. **MRT**) depends on variation either in nutrient-resorption efficiency or in longevity of different plant parts (Eckstein *et al.* 1999). Among species, leaf longevity explains more variation in **MRT** than nutrient resorption, while, within species, resorption appears to be a more relevant factor than biomass longevity (Eckstein *et al.* 1999).

In connection with a severe and rapidly increasing decline in coniferous forests, observed in Central Europe in the early 1980s, the problem of the lack of data on long-term variation in needle retention emerged. This made it impossible to compare the extent of current defoliation with the thinning of

crowns in the past (Blank 1985, Blank *et al.* 1988). In the beginning of the 1990s, a new method for retrospective assessment of needle retention on pine trees was developed (Kurkela and Jalkanen 1990). The needle trace method (NTM) is based on changes in the number of needle traces in conifers; these needle traces are embedded in the innermost tree rings, from the pith outwards, and allow the quantification of the long-term variation in needle retention on a single tree. A needle trace is defined as a part of the vascular bundle connecting the needle with the main vascular system of the shoot (Eames and MacDaniels 1935, Elliott 1937). The formation of leaf traces is closely related to the development of corresponding leaves, as shown by several studies (Larson 1975, 1984, Ewers 1982, Nelson and Dengler 1997). Therefore, it is justified to ask whether this developmental integration also allows conclusions to be drawn about leaf morphological characteristics, based on traces of the vascular bundle. Successfully linking characteristics of the needle trace with needle morphology would give valuable data on interrelations between needle longevity and needle structure. Information about changes in needle morphology during the lifetime of coniferous trees has become increasingly essential due to changes in global climate and atmospheric composition (Pearson *et al.* 1995, Woodward and Bazzaz 1995, Lin *et al.* 2001).

2. AIMS OF THE STUDY

This thesis deals with variation in needle longevity of Scots pine (*Pinus sylvestris* L.), its relation to other needle characteristics, foliar nitrogen economy, and the ageing of trees. The chronologies of needle retention in geographically distinct stands were compared in order to find regularities in needle retention pattern, which would reveal the rank of the factors of different scales, which primarily affect needle longevity. The dynamics of needles attached to the stem was investigated in relation to the ageing of trees to ascertain whether needle longevity is affected by the developmental traits of individual trees. The longevity of needles was also compared between stands of different forest types in order to establish whether small-scale variation in needle longevity is related to foliar nitrogen conservation, and whether it follows a community-specific pattern. Lastly, relationships between needle trace diameter and needle morphological characteristics were studied in an attempt to ascertain the perspectives of NTM for retrospective assessment of the changes in needle morphology.

The main questions to which answers were sought are as follows:

1. To what degree is long-term chronology of needle retention in Scots pine influenced by local factors, and to what degree by regional factors?
2. Do needle longevity and foliage dynamics depend on tree age?
3. Are longevity, specific leaf area, and nitrogen conservation of Scots pine needles interrelated and do they have a community-specific pattern?
4. Do differences in mean residence time of nitrogen result from variations in nitrogen-resorption efficiency or in needle longevity?
5. Is needle trace diameter correlated with characteristics of needle morphology?

3. MATERIALS AND METHODS

3.1. Study sites

Papers I–III: two Scots pine stands belonging to *Vaccinium* forest type were chosen for detailed analysis of long-term chronology of needle dynamics in northern Estonia. The locations were in Kose (59°19'N and 27°32'E) and in Lehtmetsa (59°12'N and 25°35'E). In co-operation with the Finnish Forest Research Institute, these stands were compared with three stands in Finland (Ruotsinpyhtää, 60°32'N and 26°27'E; Tuusula, 60°21'N and 24°57'E; Suonenjoki, 62°39'N) and with one stand in Poland (Wyszkow, 52°40'N).

Paper IV: needle longevity was estimated in 30 Scots pine stands belonging to three different forest types — *Vaccinium*, pine bog, and dry heath types (Masing 1996). In Cajander's (1926) classification, widely usable in the Nordic countries, the corresponding forest types are *Vaccinium*, pine peat moor, and *Calluna* types, respectively. The stands were located across the whole of Estonia (see map in Fig. 1 in **IV**).

Paper V: six Scots pine stands growing on two different soil types, classified by FAO-UNESCO as *Dystric Histosols* and *Podzols* (3 study sites per each soil type), were analysed for foliage nitrogen conservation and needle longevity. The stands on *Podzols* belonged to *Vaccinium* forest type and those on *Dystric Histosols,* to pine bog forest type.

Paper VI: five conifer species (*P. sylvestris*, *P. abies* (L.) Karst., *Abies sibirica* Ledeb., *Pseudotsuga menziesii* (Mirb.) Franco and *Taxus baccata* L.) were sampled in the dendrological park at Toila (59°25'N and 27°32'E), Estonia. The variation in needle trace diameter in Scots pines was measured on samples taken from 6 natural stands, 2 of them in Estonia and 4 in Finland (see map in Fig. 1 in **VI**).

3.2. Tree sampling

For revealing long-term chronologies of needle dynamics (**I–III**), twenty 40–65-year-old pines were felled according to guidelines provided by Aalto and Jalkanen (1998). The trees belonged to the main storey and were not dominated by higher trees. They had straight, unbroken stems, and their crowns were regular-shaped. Prior to felling, the eastern face of each tree trunk was marked. After felling, this mark was extended along the whole length of the stem. Discs from each tree at breast height were obtained for subsequent measurements of radial increment. The parts of the stems above breast height were sectioned into bolts corresponding to annual shoots by omitting the branch whorls as

instructed by Aalto and Jalkanen (1998). Before sectioning, the length of annual shoots was measured to the nearest cm.

For all other studies (**IV–VI**), branches were cut from the mid-part of the sample tree's crown. The height of the sample trees varied between 1.5 and 3 m. When sampling the branches, we selected only those that had no needles on at least the two oldest shoot increments. Needles from each sample branch were collected for subsequent measurements of needle morphology and nitrogen content. To characterise the growth rate of the trees in different forest types, the length of the 1-year-old shoot of the main axis was measured in all sample branches.

The number of sampled trees was 300 (10 trees per site, one branch per tree) in **IV** and 30 (5 per site, two branches per tree) in **V.** In **VI,** 22 trees (5 trees per species except *T. baccata*, which was represented by 2 trees; 10 shoots per species) were sampled for the between-species comparison, and 30 trees (5 trees per site, one or two shoots per tree) for analysing variation in needle trace diameter in Scots pine.

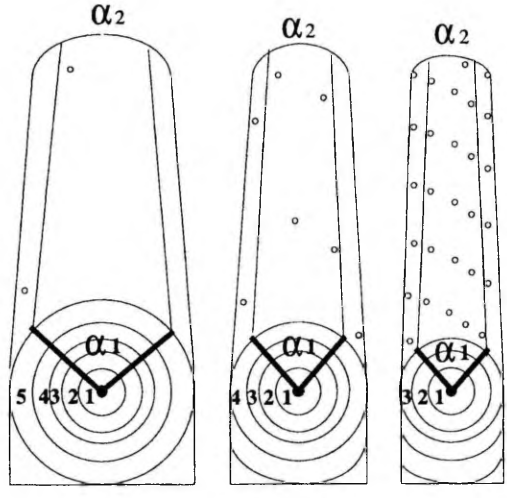

Figure 2. The needle traces appear as brown dots on planed surface. The number of needle traces was counted within the rectangle formed at the planed surface by joining the legs of the angles α_1 and α_2. (Aalto and Jalkanen 1998).

3.3. Estimation of needle retention

Long-term needle chronologies (**I–III**) were produced by means of the NTM, based on the assumptions that needle fascicles form uniformly around the stem, and that fascicle mortality is evenly distributed irrespective of compass direction (Kurkela and Jalkanen 1990). A detailed description of the NTM and the computational technique has been compiled by Aalto and Jalkanen (1998). To test the assumptions of the NTM, we counted living needle fascicles on the same shoots of the sample trees that were used for analysing long-term chronology of needle retention.

In the laboratory, the calendar year of shoot formation was marked on each annual bolt. Lines at angles of 40°–180°, depending on thickness of the growth rings, and emanating from the pith were marked at the ends of the bolts (Aalto and Jalkanen 1998). The bolts were planed along the eastern side, ring by ring, towards the pith. For each of the 5–6 innermost rings, the ends of the angled lines, where they met the planed surface at either end of the bolt, were joined by lines, and the number of needle traces within the rectangle formed at the planed surface was recorded (Fig. 1). The survivorship of needles on the shoot was estimated by dividing the number of traces in a particular ring of the shoot by the number in the innermost ring. Such a definition of needle survivorship is analogous to that used by Whitney (1982) and Nilsen *et al.* (1987). Proceeding from the survivorship, we calculated annual needle retention ($\mathbf{NR_t}$) and the mean longevity of needles (**A**, years) attached to the particular shoot as:

$$NR_t = \frac{\sum[x_t,(x-1)_t...(x-n)_t]}{100}, \qquad (1)$$

where $\mathbf{x_t}$ is the survivorship of needles on shoot **x** present in year **t**, $\mathbf{(x\text{-}1)_t}$ is the survivorship of needles in year **t** on the shoot initiated the year before shoot **x**, and so on.

$$A = \sum_{r=1}^{i}\left[(x_r - x_{r+1})\left(r-1+\frac{m}{12}\right)\right], \qquad (2)$$

where $\mathbf{x_r}$ is the survivorship of needles on the bolt in the year corresponding to the formation of the ring **r**, and **m** is the number of months between the birth of new needles and the yellowing of the oldest needles (taken as 3 months in this study). For computing needle production (**P**), i.e. the number of needle fascicles attached to the shoot in the year of shoot formation, we used the following equation:

$$P = \left(\frac{n \cdot 360^{\circ}}{l_b \cdot \alpha} \right) \cdot l_s \quad , \tag{3}$$

where **n** is the number of needle traces in the innermost ring, $\mathbf{l_b}$ is the length of the corresponding bolt, $\mathbf{l_s}$ is the length of the corresponding shoot, α is the mean of the angles drawn at the ends of the bolt. The number of needles on a shoot, in any year after the shoot formation, was obtained by multiplying the needle production of the shoot by needle survivorship. We were then able to estimate the overall number of needle fascicles (**N**) attached to the stem of the tree in a particular year, as:

$$N = \sum_{i=1}^{n} f_i \quad , \tag{4}$$

where $\mathbf{f_i}$ is the number of fascicles on the $\mathbf{i^{th}}$ shoot of the stem. Shedding of needles (**S**), i.e. the number of needle fascicles shed annually from the main stem of the tree, was calculated as follows:

$$S = (N_t - N_{t+1}) + P_{t+1} \quad , \tag{5}$$

where index **t** is the year number. Finally, we found the quotient of needle loss, by dividing needle shedding by needle production in the same year (Nebel and Matile 1992).

On sample branches, maximum needle longevity was estimated by counting the number of needle age classes on the main axis of each branch (**IV–V**). The survivorship of needles in each needle age class (**IV**) was estimated according to Whitney (1982), Nilsen *et al.* (1987) and Aerts (1989), by dividing the number of living needles by the sum of the number of needle scars and living needles. By dividing the sum of needle survivorship by the number of needle age classes, the mean survivorship of needles was calculated. This parameter is not related to any certain needle age class, but reflects the average level of survivorship and is independent of the actual number of needles. Hence, its use is justified if needle survivorship is compared among habitats where needle production is different, but where maximum needle longevity is similar.

3.4. Needle morphometric measurements

Needle morphology was characterised by three directly measurable morphometric variables (needle length, width, and thickness) measured on freshly collected needles. The width and thickness of needles was measured with a precise caliper (±0.01 mm) at the top, central, and basal parts of the needles. Needle length was measured with a ruler, and the total surface area was cal-

culated as the product of needle circumference and length, assuming the needles to be half-cylinders (Berninger and Nikinmaa 1994) or the needle cross section to be elliptic (Niinemets *et al.* 2001). The dry mass of the sampled needles was determined after drying at 65°C for 48 hours, and needle dry mass per unit total area (**LMA**, g m^{-2}) and its reciprocal (**SLA**, m^2 g^{-1}) were calculated (**IV–V**).

Diameter of the needle traces ($\mathbf{D_{nt}}$) was measured in the last growth ring of the wood samples for inter-specific comparisons, and in the second outermost tree ring for comparisons between Scots pines (**VI**). The diameter was measured at right angles to the longitudinal axis of the long shoots under a stereomicroscope at 50× magnification. In the case of Scots pine, some needles were additionally studied to find stomatal density by counting stomata on the adaxial surface of the needles.

3.5. Analysis of foliar nitrogen economy

For analysing mass-based nitrogen concentration ($\mathbf{N_m}$, mg g^{-1}) and nitrogen conservation in foliage, we collected needles three times during a growth period. We sampled green needles from the oldest needle age classes (referred to as **Set 1**) in June, just after the sprouting of new needles. The current-year needles were collected at the end of July (**Set 2**), and the yellowing needles from the oldest age classes just before abscission, in September (**Set 3**). The $\mathbf{N_m}$ was assessed according to Kjeldhal's method at the Plant Biochemistry Laboratory of the Estonian Agricultural University (Tartu, Estonia).

Nitrogen conservation was characterised by nitrogen-resorption efficiency, proficiency, and mean residence time (Killingbeck and Whitford 2001). The nitrogen-resorption efficiency (**NRE**) is the percentage of the element removed from senescent leaves, and it was calculated as follows:

$$NRE = \frac{(N_{m1} - N_{m3}) \cdot 100}{N_{m1}} \quad , \tag{6}$$

where $\mathbf{N_{m1}}$ and $\mathbf{N_{m3}}$ are leaf nitrogen concentrations (mg g^{-1}) of **Sets 1** and **3**, respectively. The nitrogen-resorption proficiency (**NRP**) is the level to which a nutrient has been reduced in the senescent leaves, and here is expressed as the concentration (%) of nitrogen in **Set 3**. The mean residence time of nitrogen (**MRT**) was calculated according to Eckstein et al. (1999):

$$MRT = \frac{N_{m2} \cdot A_{max}}{N_{m3}} \quad , \tag{7}$$

where $\mathbf{N_{m2}}$ is leaf nitrogen concentration (mg g^{-1}) of **Set 2**, $\mathbf{A_{max}}$ is maximum needle longevity (years).

3.6. Statistical analyses

Differences in needle retention characteristics between the Estonian and Finnish study stands were investigated by means of the paired *t*-test at significance level α=0.05 (**I**). Pearson's correlation coefficient was used for estimating the strength of similarities in needle chronologies between the two countries (**II**). For analysing the effect of trees' ageing on needle dynamics (**III**), the data was arrayed according to tree age at breast height. It was arbitrarily assumed that 1.3-metre-tall trees were one year old. For each age, we calculated means of the variables and discarded those ages represented by fewer than five sample trees. In the subsequent analysis, we applied linear regression to describe long-term trends in the mean values of the characteristics. To discover any short-term regularity, we applied a one-year time lag to the data and found the partial autocorrelation coefficients for the transformed data.

To compare the effects of sampling sites and forest types on the number of needle age classes, mean survivorship of needles, and length of shoots, a mixed model of two-level nested analysis of variance (ANOVA) was applied (**IV**). Sampling site was treated as a random factor nested within forest type (a fixed factor). Statistical comparisons of the means of $\mathbf{N_m}$ and **SLA** among forest types were conducted by applying a *TYPE I* one-way ANOVA. To compare needle survivorship of the same needle age class, we first calculated mean values for each sampling site and then compared them using a Kruskal-Wallis test with forest type as a grouping variable.

A mixed model of two-level nested ANOVA was also used to compare the effects of sampling site and soil type on needle longevity, $\mathbf{N_m}$, **NRE**, **NRP**, **MRT**, needle surface area, and **LMA** (**V**). Sampling site was treated as a random factor nested within soil type (a fixed factor).

Three-way nested ANOVA was used for estimating the variation in needle trace diameter among species, among shoots within a species, and among needles within a shoot (**VI**). All factors (species, shoot and needles) were treated as random. For comparing needle traces within Scots pine, 3-way nested ANOVA was used for estimating variation among sites, shoots and needles. All three factors were treated as random.

The assumptions of normality and homogeneity of variances were checked using the Kolmogorov-Smirnov *D*-statistic and the Brown-Forsythe test, respectively. *Post hoc* mean comparisons were performed using the Student-Newman-Keuls multiple range procedure. All statistical computations were made with the aid of the computer package Statistica'98 (StatSoft Inc., Tulsa, USA) at the significance level α=0.05 (Statistica 1998).

4. RESULTS

4.1. Validation of the needle trace method

Recent tests of the assumptions of the NTM, carried out by other researchers (Jalkanen *et al.* 2000, Pouttu and Dobbertin 2000) on pines, have confirmed a strong correlation between the estimates and actual needle retention data obtained by counting living needle fascicles on the same shoots. In the present study, the survivorship of pine needles established by NTM was not significantly different from the actual survivorship obtained by needle counting, if the means of all sample trees were compared (Fig. 2a). In single trees, however, the results of the NTM may differ from the actual survivorship of needles. For example, the biggest difference in the needle survivorship established by NTM and by needle counting was 0.37. That difference, which tended to increase with needle age, indicates that needle shedding is not even throughout the whole shoot. This is confirmed by the fact that there was no difference between the actual survivorship and that established by using NTM for the wholly foliated shoots (i.e. survivorship of needles = 1).

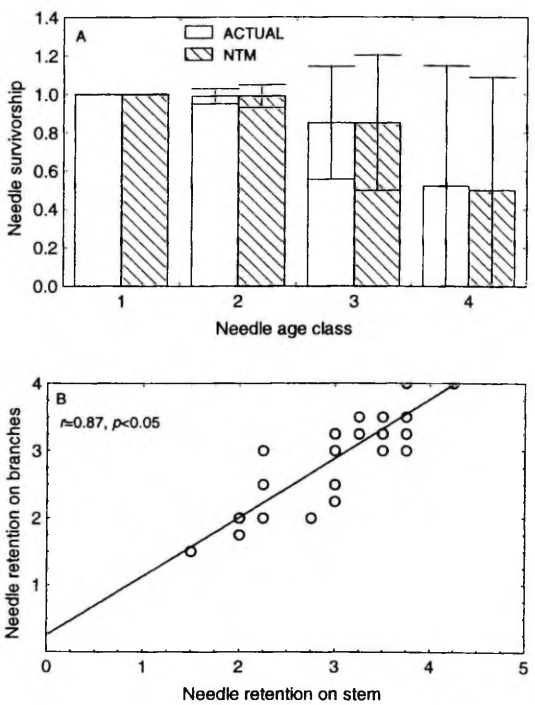

Figure 2. (A) Comparison of the survivorship of needles obtained by NTM and by counting the actual number of needles on shoots of Scots pine. The error bars indicate 1.96×SD. (B) Needle retention on the main stem versus branches.

The assumption that the characteristics of needle retention obtained on the stem can be transferred to branches is proved by the high correlation (r=0.87, p<0.05) between the respective data (Fig. 2b). Jalkanen *et al.* (1995) also observed a strong correlation (r=0.78) between the needle retention on stem and branches for Scots pine trees growing in Finland.

4.2. Long-term needle retention chronology

Annual average needle retention values ($\mathbf{NR_t}$) varied from just above 3.1 to 2.2 needle sets in the Estonian, and between 3.1 and 4.2 in the Finnish stands (Fig. 1a in **I**). The 25-year-mean of 20 trees in Estonia was significantly (p<0.001) lower than that in Finland. The long-term difference in needle retention between the sample stands was not significant for either Finland or Estonia. From 1964 to 1986, average standwise needle age (**A**) varied annually between 2.6 and 3.4 years in Finland, and between 1.5 and 2.3 years in Estonia (Fig. 1b in **I**). The mean needle age for this period was significantly (p<0.001) higher in Finland (arithmetic mean ± SD, 3.0±0.2) than in Estonia (2.1±0.2). In both countries, the minimum in average needle age occurred at the beginning of the 1980s. The synchronicity in the short-term pattern of needle retention chronologies between the two countries was supported by highly significant correlation (r was up to 0.7 in **II**). In the stand in Poland, the patterns of the $\mathbf{NR_t}$ and **A** differed from those of the Estonian or Finnish stands (**II**). There were no significant correlations between the Polish data and that of the more northern stands.

4.3. Ontogenetic trends in long-term needle dynamics

All variables used for characterising needle dynamics were related to the ontogenetic development of the trees (**III**). Mean needle longevity increased slightly with tree age, although it was very variable from tree to tree. For the Finnish sample trees, the average level of **A** increased significantly from 2.7±0.1 (arithmetic mean ± SD) years at age one, to almost 3 years at age 38 (Fig. 1 in **III**). As for the Estonian stands, the increasing trend was not statistically significant. Comparison of different age classes revealed that survivorship of the needles of the current and two previous years did not change, while survivorship in the fourth age class increased significantly with the ageing of the trees. Moreover, the older the trees, the more frequently even 4-year-old needles were present on shoots (Fig. 2a in **III**).

The mean needle production (**P**) fluctuated around a mean level of 240±4 needle fascicles per shoot from age 1 to age 51 in Estonia (Fig. 2b in **III**), after which it declined sharply to 100 fascicles by age 62. There was a significant negative autocorrelation (r=-0.37, p<0.05) between **P** of consecutive years,

which suggests that years with a high level of **P** were followed by years with remarkably lower production. The mean overall number of needle fascicles (**N**) at the stem was approximately 684±11 for trees aged 4 to 51 years, followed by a sharp decrease in older trees (Fig 2b in **III**). The partial autocorrelation of the time-lagged data was insignificant, suggesting that **N** did not alternate regularly in a short-term time scale.

Mean needle shedding (**S**) fluctuated around the same level (240±9) as **P**, but the dispersion of the data was greater (Fig. 2b in **III**). The breakpoint occurred at the age of 50 years, after which **S** began to decline sharply. Like **P**, the time-lagged data of **S** showed a regular alternation of high and low levels from year to year (r=-0.58, p<0.05). The mean quotient of needle loss (defined as **S/P**) tended to increase slightly as the trees become older (Fig. 2c in **III**). When the quotient of needle loss was transformed by applying a one year time lag, a significant short-term pattern was revealed: greater quotients were followed by smaller ones (r=-0.65, p<0.05).

4.4. Community specific variation

In the Estonian stands growing in different forest types, the maximum age of needles averaged 4 years (**IV**). The variation in maximum needle age among sampling sites within the forest types and among individual trees within the sites was highly significant (p<0.001), whereas the effect of forest type was statistically insignificant (Table 2 in **IV**, Table 2 in **V**). Contrary to the effect of forest type on the maximum age of needles, its effect on mean needle survivorship was significant (p<0.01), while that of sampling sites within forest types was statistically insignificant (Table 2 in **IV**).

The overall average of the needle survivorship in *Vaccinium* forests was significantly (p<0.05) smaller than in the other two forest types (Table 3 in **IV**). The survivorship of the current-year and 1-year-old needles was equal in all forest types, but that of the 2- and 3-year-old needles was significantly (p<0.05) smaller in *Vaccinium* forests (Fig. 2 in **IV**). The 4-year-old needles demonstrated the same survivorship in all forest types, but the needles of the trees growing in *Vaccinium* forests had a greater chance of living 5 years. Thus, the differences in the mean needle survivorship between the forest types were due to the smaller survivorship of 2- and 3-year-old needles in *Vaccinium* forests, whereas the decline in the mortality rate of the 4-year-old needles resulted in the same number of needle age classes across all the forest types.

4.5. Needle morphology and retention

Surface area of the needles varied significantly (p=0.003) between the forest types, but not among sampling sites (Table 2 in **V**). Pines growing in the bog forest type (on organogenic soil) averaged 1.6 times smaller needle surface area than pines in *Vaccinium* forest type (on mineral soil) (**V**). The mean values of **SLA** for dry heath and *Vaccinium* forests were similar and significantly higher (p<0.05) than the value for pine bog forests (Table 3 in **IV**). Needle mass per area (**LMA**) did not differ significantly either between soil types or among sampling sites, ranging from 50 to 200 g m^{-2} (Table 2 in **V**). Maximum needle longevity was negatively correlated (r=–0.61, p<0.05) with **LMA** (**V**).

The forest types explained approximately 28%, and sampling sites about 16%, of the total variation in the shoot length increment (Table 2 in **IV**). The mean shoot length increment in pine bog forests was significantly (p<0.05) smaller than that in *Vaccinium* and dry heath forests. When all the data was pooled, the shoot length increment showed a weak negative but statistically significant (r=–0.13, p<0.05) correlation with needle longevity (**IV**).

4.6. Foliar nitrogen content, nitrogen conservation and needle longevity

Nitrogen concentration ($\mathbf{N_m}$) of the needles depended on needle age, forest type, and sampling site (Table 3 in **IV**, Table 3 in **V**). Like the mean needle survivorship, $\mathbf{N_m}$ of the needles collected from the *Vaccinium* forests differed from that in the pine bog and dry heath forests (Table 3 in **IV**). The interaction of needle age and forest type also significantly (p<0.001) affected $\mathbf{N_m}$ (Table 3 and Fig. 2 in **V**). Within forest types, the $\mathbf{N_{m3}}$ (4.4±0.5 mg g^{-1} in the *Vaccinium* and 3.5±0.7 mg g^{-1} in the pine bog forest type) was significantly lower (p<0.05) than $\mathbf{N_{m1}}$ and $\mathbf{N_{m2}}$ (Fig. 2 in **V**). $\mathbf{N_{m3}}$ did not significantly differ between the two forest types. In the pine bog forest type, the $\mathbf{N_{m1}}$ (8.1±1.3 mg g^{-1}) did not differ from that of $\mathbf{N_{m2}}$ (7.2±1.1 mg g^{-1}), while both were significantly (p<0.05) smaller than the $\mathbf{N_m}$ of green needles in the *Vaccinium* type (Fig. 2 in **V**).

Nitrogen conservation was studied in the *Vaccinium* and in the pine bog forest types. Trees growing in the *Vaccinium* type exhibited nearly 10% higher nitrogen-resorption efficiency (**NRE**) as compared to trees in the pine bog forest type (p=0.002, **V**). Nitrogen-resorption proficiency (**NRP**) did not significantly differ between the forest types. As with **NRE**, the effect of sampling site on **NRP** was not statistically significant (Table 4 in **V**). Mean residence time of nitrogen (**MRT**) in pine needles was 8.4±2.3 years for the stands in the *Vaccinium* type and 6.1±1.2 years for those in the pine bog forest type. Nevertheless, the differences in **MRT** between the different forest types turned out to be insignificant (Table 4 in **V**).

The $\mathbf{N_{m2}}$ was positively correlated with $\mathbf{N_{m1}}$ (r=0.89, p<0.05) and $\mathbf{N_{m3}}$ (r=0.61, p<0.05; Table 5 in **V**). The greater the surface area of the needles, the higher was the $\mathbf{N_m}$ of the green leaves (r=0.76, p<0.05). Mean residence time was positively related to maximum needle age (r=0.77, p<0.05; Fig. 3 in **V**) and needle area (r=0.63, p<0.05).

4.7. Relationships between needle-trace diameter and needle morphology

The diameter of needle traces ($\mathbf{D_{nt}}$) varied significantly among species (p<0.001), as well as among shoots of the same species (p<0.001), but not among needles on the same shoot (Table 1 in **VI**). Among the species studied, Scots pine had the biggest $\mathbf{D_{nt}}$ (arithmetic mean ± SD, 233±30 μm), followed by *T. baccata* (141±19 μm) and *P. menziesii* (121±30 μm). *Picea abies* and *A. sibirica* had the smallest $\mathbf{D_{nt}}$ (80±18 and 85±21 μm, respectively. Fig. 3 in **VI**). Across all species, the diameter of needle traces was strongly correlated (r=0.93, p<0.05) with needle length (Fig. 4a in **VI**). Correlation between the $\mathbf{D_{nt}}$ and other morphometric variables (needle width and thickness) was not significant.

The diameter of needle traces of Scots pine varied significantly (for all factors p<0.001) among sampling sites, among shoots within the sites, and among needles on the same shoot (Table 2 in **VI**). The measured needle characteristics, except needle thickness, demonstrated a significant positive correlation with $\mathbf{D_{nt}}$ in pine. The strongest relationship (r=0.64, p<0.05) was revealed between the mean $\mathbf{D_{nt}}$ and needle dry weight (Fig. 4b in **VI**). The correlation between the diameter of needle traces and the average length of needle pair was weak, but still significant (r=0.35, p<0.05). The correlation coefficient for the relationship between $\mathbf{D_{nt}}$ and the average needle width was 0.55 (p<0.05; Fig. 4c in **VI**). Stomatal density was positively related to $\mathbf{D_{nt}}$ (r=0.55, p<0.05; Fig. 4d in **VI**).

5. DISCUSSION

5.1. Long-term chronology of needle retention

The differences in mean value of needle retention revealed between the Estonian and Finnish Scots pine stands seem to be in good agreement with the latitudinal variation in needle longevity shown by Pravdin (1964) in Russia and by Jalkanen *et al.* (1995) in Finland. The correlation between needle longevity and latitude is related to the latitudinal variation in thermal sums (Jalkanen *et al.* 1995) or January temperatures (Xiao 2003). The impact of thermal conditions on needle retention indicates the important role of climate (summer and winter temperatures) in determining needle longevity. The mean needle age that was observed in this study (**I, II**) is less than the average longevity of pine needles recorded by other researchers in Estonia. Tullus (1991) as well as Drenkhan and Hanso (2003) have shown that needle longevity of Scots pine fluctuates around 3.0 years. That difference is most probably caused by the fact that needle longevity is a very variable characteristic, which varies even among trees growing in the same stand (**IV, V**), and bigger sample sizes are therefore needed in order to draw reliable conclusions about whole of Estonia.

Short-term fluctuations in needle longevity were recorded in all experimental stands. To discover the reasons for this variation, it is necessary to examine the factors influencing the mean age of needles formed in particular years. Mean needle age fluctuated at an interval of 2–7-years; this also brought about corresponding fluctuations in needle retention and needle loss (Fig. 1 in **I**). This short-term variation can not be caused by silvicultural practises, because the intervals between the peaks are too short, and in Estonia the stands had not been thinned before the mid 1990s. The strong correlation between needle age chronologies in Estonia and Finland suggests that the factors causing the short-term variation are of a regional rather than local nature. A drastic decrease in needle longevity occurred at the beginning of the 1980s. In all the Estonian and Finnish study stands, the needles born in this period had a significantly shorter life than the long-term average needle life-span (Fig. 1b in **I**). Weather is certainly one of the primary factors that has an important role in determining the short-term variation in needle longevity. However, the sharp decrease in mean needle age in the first half of the 1980s can hardly be explained merely by weather conditions. The decline in mean needle age lasted 2–3 successive years, but there was no such steady trend in weather conditions either in Estonia or in Finland at that time. Although, if the circumstances for carbon assimilation are considered — extremely high rainfall in 1978 and 1979, and therefore also a low level of irradiance — might cause the rate of net photosynthesis to drop. This, in its turn, might influence the condition and hardiness of the needles born in those years and later. However, the decrease of mean needle age in the early 1980s, observed in this study, was nearly simultaneous with the widespread damage of coniferous

forests in western Europe, making it likely that both events had the same cause. In Western Europe, the forest damage was thought to be a direct consequence of air pollution (Blank 1985). The spreading of air pollutants over long distances in combination with weather fluctuations could also have induced the decrease in needle longevity in the areas under discussion.

5.2. Ontogenetic trends in long-term needle dynamics

Seiwa (1999a,b) has discussed the possibility that the differences in leaf longevity between seedlings and mature trees in *Acer mono* Maxim. and *Ulmus davidiana* var. *japonica* Nikai may partly be due to ontogenetic changes. The long-term shift in the needle longevity of *Pinus sylvestris* (**III**) was an age-dependent phenomenon rather than a consequence of changing environmental conditions. Although mean needle longevity was very variable from tree to tree, its average level and the survivorship of older needles tended to increase with tree age. Recently Xiao (2003) published similar results also for *Pinus tabulaeformis*. One probable reason for this ontogenetic trend may be changes in water supply for the needles of older or taller trees, limiting stomatal conductance and photosynthesis (Ryan and Yoder 1997, Hubbard *et al.* 1999, Kolb and Stone 2000). Since the height of the sample trees varied from 1.3 m up to almost 19 m, there should have been remarkable differences in the gravitational component of the leaf water potential across the height range of the sampled trees, given as a water potential decline by 0.01 MPa m^{-1} (Nobel 1991). In fact, shoot water potential may decrease even more steeply with tree height, especially in conifers, because of the high frictional resistance of their xylem to water flow, which is proportional to the length of the transport pathway (Tyree and Ewers 1991).

Hydraulic conductance of Scots pine has been reported to be greatest at an age of 15–20 years, and to decrease in older trees (Mencuccini and Grace 1996). Annual biomass growth per unit leaf area (growth efficiency) declines with tree age, whereas aboveground sapwood volume per unit leaf area, which is related to maintenance respiration costs, steadily increases. Mencuccini and Grace (1996) suggested that increased hydraulic resistance and maintenance respiration costs might be the main causes of reduced carbon gain in mature and old pine trees. Recently Magnani *et al.* (2000) proposed a model to explain the age-related decline in productivity of a pine stand. According to the model, resource allocation is predicted to shift from foliage to transport tissues, most notably to fine roots, with increasing tree age. Both higher respiration costs and fine root turnover would result in a decline in net primary productivity. Thus, the increase in needle longevity and survivorship in taller/older pine trees may reflect a more economical use of resources under conditions of diminishing net assimilation rates.

The decline in needle production observed in pines older than 50 years may also be a sign of the negative influence of increased hydraulic resistance on tree growth. At the same time, needle shedding also declined. In young trees the mean rates of needle production and shedding did not vary with tree age, but at an age above 50 years the decrease in needle loss was slower than that in needle production. Thus, the quotient of needle shedding of trees older than 50 years tended to be over 1.0, indicating that the number of needles shed in autumn exceeded the number of needles produced in the previous spring. Therefore, the overall number of needles decreased faster than one would expect from the slope of the needle production curve. Owing to the increase in needle longevity and the decrease in needle production, older trees had relatively more needles of the third and fourth age classes as compared to younger trees, but, in absolute numbers, they still had fewer needles per stem.

Needle production, shedding, and quotient of needle loss exhibited regular short-term fluctuations. Larger values of these characteristics were followed by smaller ones in the next growing season. Such regularity is difficult to explain. One explanation might be analogous to that of the fluctuations of any physiological parameter or population density around an equilibrium value, due to the homeostatic mechanisms that operate in biological systems. The degree of shading along the branches has been shown to determine the amount of foliage in the crown of *Pinus contorta* ssp. *latifolia* (Engelm.) (Schoettle and Smith 1991). The concept of ecological light compensation point (Schoettle and Fahey 1994) suggests that leaves have critical light requirements, below which they cannot maintain a positive carbon balance and are to be lost. As the tree produces more needles in one spring, the degree of shading may increase. This may cause the carbon gain of older foliage to drop below the critical value, which brings about both increased needle mortality in autumn and decreased needle production in the following spring. Thus the tree can maintain the overall number of needles at a more-or-less constant level, which is optimal for carbon assimilation in the given circumstances.

5.3. Community-specific pattern in variation of needle longevity

The results of the study (**IV**) confirmed that mean needle survivorship in Scots pine is smaller in plant communities formed on fertile sites (*Vaccinium* forests) as compared to communities on sites with limited resource availability (dry heath and pine bog forests), whereas the maximum needle longevity did not significantly differ between the communities. However, in studies on intraspecific variation of conifers' needle longevity across climatic zones, it has been shown that the maximum longevity of needles is also variable (Ewers and Schmid 1981, Schoettle 1990, Nebel and Matile 1992). We suppose that within the same climatic zone, Scots pine needles may achieve equal maximum age in different

habitats, but that the mean survivorship of needles tends to be greater in infertile growth sites. The fertilising experiment performed by Aerts (1989) gave similar results for *Erica tetralix* L.: adding of nutrients caused the leaf survivorship to decrease, whilst the maximum leaf longevity did not change. Consequently, the intra-specific variation of leaf longevity in evergreens is much less within the same climatic zone than it is across different climatic zones.

Inter-specific comparisons have indicated that woody species with smaller leaf longevity have a greater $\mathbf{N_m}$ (Reich *et al.* 1997, 1998a, 1999, Eamus *et al.* 1999, Lusk and Contreras 1999, Kloeppel *et al.* 2000) and **SLA** (Tobin *et al.* 1999, Warren and Adams 2000) than species with longer leaf longevity under the same growth conditions. This relationship seems to be of a universal nature since the same trends both in $\mathbf{N_m}$ and **SLA** with respect to leaf longevity have been revealed for perennial herbs as well (Diemer 1998). Intra-specifically, increased needle longevity of Scots pine at higher latitudes or altitudes seems to be phenotypic acclimation to low temperature and nutrient availability, which may be accompanied (but not necessarily) by smaller $\mathbf{N_m}$ (Reich *et al.* 1992, 1996a, 1996b). We suppose that greater needle survivorship of Scots pine trees in dry heath and pine bog forests reflects higher resource-use efficiency, and thus can be considered as an acclimation to low nutrient availability. The same foliage is usable for a longer time period and plants have to spend fewer resources to form new leaves. Besides, there is a greater selective pressure in nutrient-poor habitats to maximise the duration of nutrient retention in leaves (Aerts and van der Peijl 1993). Thus, our results are in accordance with those of Aerts *et al.* (1999), who found that the $\mathbf{N_m}$ of the evergreen species from temperate fens and bogs was lower, and nitrogen-use efficiency was higher, than those of the evergreens in fertile environments. Enoki and Kawaguchi (1999) also related intra-specific variation in needle longevity of *Pinus thunbergii* Parl. to nutrient-use efficiency.

Despite the water-saturated soil, bog-growing trees may often even suffer from dynamic water stress resulting from weak stomatal control of transpiration, bringing about large water losses from the foliage and low leaf water potential in the midday period of warm and sunny summer days (Sellin 2001). Thus, in the case of the pines growing in bog forests, more frequent dynamic water stress and higher construction cost (indirectly indicated by needle dry weight to area ratio, **LMA**) of the needles may also contribute to the increase in mean needle longevity.

In summary, the extended needle survivorship of Scots pine in conditions of low resource availability probably has the same ecological significance as the distribution of evergreen and deciduous species along soil fertility gradients: species with long-living foliage have advantages in nutrient-poor environments (Monk 1966, Bazazz 1979, Kloeppel *et al.* 2000). For pine trees, a longer period of leaf retention in infertile sites most likely confers higher resource-use efficiency, which is needed to achieve a positive carbon balance at limited resource availability.

5.4. Relationships between needle morphology and needle retention

The three forest types considered in the present study were ranked in the same order in respect to both the N_m and the **SLA** of Scots pine (**IV**): both characteristics were the highest in *Vaccinium* forests, intermediate in dry heath forests, and lowest in pine bog forests. It has been shown for many species that photosynthetic capacity or relative growth rate are closely related to both **SLA** and N_m (Reich *et al.* 1998b, Wright and Westoby 1999, 2000, Rijkers *et al.* 2000, Wright *et al.* 2001). According to the photosynthesis — nitrogen-use relationships found from inter- and intra-specific comparisons (Gower and Richards 1990, Reich *et al.* 1998a, Poorter and Evans 1998), and the corresponding patterns in N_m and **SLA**, we suppose that the net photosynthetic capacity of pines is higher in *Vaccinium* forests, intermediate in dry heath forests and lower in pine bog forests. Although both the surface area and weight of the needles were smaller, the **SLA** of the trees growing in pine bog forests was still significantly lower compared to the trees in the other two forest types. Thus, the trees growing in waterlogged and infertile soil in pine bog forests invest more biomass per unit area of photosynthetic tissue, which has been shown to be a characteristic of leaves in arid environments (Reich *et al.* 1999, Bussotti *et al.* 2000). Wright and Westoby (1999), exploring variations in relative growth rate and morphological traits of woody dicotyledonous species in respect to nutrient availability and rainfall, concluded that the gradients of decreasing nutrients or rainfall actually appear to be variants of a more general 'stress' gradient. Their results and those published by Poorter and de Jong (1999), and Castro-Diez *et al.* (2000) confirmed the same trend that was revealed for Scots pine in the present study: **SLA** decreased with decreasing resource availability or increasing stress level. In the present case, low nutrient availability and hypoxia are the stress factors combined in waterlogged bog soils. Decrease in **SLA** in response to low nutrient availability can be considered to be a universal phenomenon, common for different life forms. Meziane and Shipley (1999) demonstrated the same regularity in their experiments with 22 herbaceous species.

Schoettle (1990) has shown that an increase in needle longevity of *Pinus contorta* Dougl. ex Loud. with the altitude of the growth site was accompanied by decreasing shoot length increment. In this study we found that, despite longer needle longevity, the annual shoot increments of the trees in dry heath forests did not differ from those of the trees in *Vaccinium* forests. Nevertheless, when all data was pooled, the shoot length increment was negatively correlated ($p<0.05$) with needle longevity.

Needle traits also differ significantly depending on soil conditions. Our study revealed that needle surface area was 1.6 times smaller for trees growing on organogenic soil (*Dystric Histosols*) compared to trees on mineral soil

(*Podzols*). This tendency is probably related to both low nutrient availability and hypoxia, resulting from waterlogged conditions, in *Dystric Histosols*. The stands growing in the two sites with differing soil conditions did not differ significantly in **LMA** (**V**). This contradicted our previous data, which indicating that specific leaf area (i.e. inverse of **LMA**) of pine needles declined with decreasing site fertility (**IV**). The disagreement probably resulted from the high variability of **LMA** between sites and trees, and the fact that only three stands per soil type (versus 10 sites per forest type in **IV**) were sampled in **V**.

The positive relationship between needle longevity and needle area, as well as the negative correlation with **LMA**, are somewhat surprising results in the light of the theory of stress resistance syndrome (SRS), which states that, in infertile habitats, plants have scleromorphic leaves, slow growth rate, low photosynthetic rate, and low leaf turnover rate (Chapin *et al.* 1993). The pine trees growing under stress conditions (low growth rate on waterlogged organogenic soil) had needles with smaller surface area, which, according to SRS, should be accompanied by longer leaf life-span and higher **LMA**. However, the correlation analysis showed that the greater the needle surface area (which was more likely in the case of the pines on the mineral soil), the longer the needles' life span tended to be. As the links between leaf structure, leaf longevity, and stress resistance have been found mostly in inter-specific comparisons (Chapin *et al.* 1993, Reich *et al.* 1992), the intra-specific relationships may be of a different nature. Hence, Eckstein and Karlsson (2001) could not find a trade-off between **MRT** and nutrient productivity within species, although it was observed between species. The variation of leaf traits resulting from SRS within a single species is probably too small to be exhibited as measurable relations between leaf properties, while at the inter-specific level, the expected relationships are likely to be more pronounced, since wider ranges of values are covered. The positive correlation between needle surface area and their maximum longevity, observed in this study, is most likely caused by the fact that bigger needles are characteristic of healthy, well-growing trees which are able to retain their foliage for a longer period. However, more studies need to be carried out on different species that are distributed across a wide range of habitats in order to clarify the intra-specific relationships between leaf life span, leaf morphology, and nutrient use strategy.

5.5. Nitrogen conservation

Scots pine is a species able to cope with severe shortages of nutrients and water or with an excess of soil moisture. According to Grime (1977) it belongs to a stress-tolerant strategy type, reassessed by Grubb (1998) as a switching strategy type. As Scots pine occupies a wide range of habitats, the trees vary in pheno-

type. For example, pines growing in infertile sites are rather small and exhibit low growth rates, while, in fertile sites they are large and fast growing trees.

Foliage nitrogen concentration, expressed per mass of green needles, was clearly greater in trees on mineral soil, being the highest in mature green needles ($\mathbf{N_{m1}}$). On organogenic soil, the current-year needles had the same level of nitrogen concentration as old green needles, but on mineral soil the nitrogen concentration was higher in old green needles than in current-year needles. Studies concerning the nutrient content of different aged foliage have confirmed that current-year needles have the highest nitrogen level (Florence and Chuong 1974, Hom and Oedul 1983, Nambiar and Fife 1987). Therefore it is most plausible that current year needles (**Set 2**) had not achieved the maximum value of $\mathbf{N_m}$ by the end of July, when they were sampled.

Trees growing on mineral soil were more efficient at resorbing nitrogen from senescent needles than trees growing on the organogenic soil. Mean nitrogen-resorption efficiency in foliage for the pines growing on the mineral and organogenic soils (65% and 56%, respectively) was consistent with most of the data published by other authors. In the study carried out by Näsholm (1994) on Scots pine, the **NRE** varied between 45 and 65%, and in that by Helmisaari (1992), between 40 to 70%. In studies carried out on other pine species, **NRE** varied between 33 and 62% (Dalla-Tea and Jokela 1994, Escudero *et al.* 1992, Nambiar and Fife 1987). In different studies across a wide range of evergreen shrubs and trees, the NRE in foliage averaged 47% (Aerts 1996) and 34% (expressed on a leaf area basis, Wright and Westoby 2003).

Nitrogen-resorption proficiency of Scots pines was similar for both soil types: on average 0.44% for *Podzols* and 0.35% for *Dystric Histosols*. These values were remarkably smaller than mean nitrogen concentrations in the senescent leaves of many deciduous and evergreen woody species, published by Chapin and Kedrowski (1983; 0.82%) and by Killingbeck (1996; 0.87%). Using the NRE estimations published for Scots pine (Escudero *et al.* 1992, Näsholm 1994), Killingbeck (1996) found the **NRP** to be 0.50–0.52%. Our findings indicate that Scots pine is able to reduce nitrogen to even lower concentrations in its senescent needles, and complete resorption of nitrogen (ultimate resorption proficiency, defined as a maximum level to which nitrogen can be reduced in senescent leaves; Killingbeck 1996) apparently took place on both soil types. Killingbeck (1996) has stated that **NRP** appears to be an especially useful term for addressing questions related to the selection pressures that have influenced the nutrient resorption process in plants. Nutrient-resorption proficiency values are more definite and objective measures of the degree to which plants can minimise nutrient loss. Nitrogen-resorption efficiency values are better suited to describing the relative degree to which plants can conserve nutrients invested in foliage. It encompasses both nutrient demand and nutrient withdrawal.

Scots pine, as a light-demanding species, is distributed on a wide variety of soil types, being restricted on more fertile sites by fast-growing tree species that are better competitors for light. However, its ability to manage with a low level

of nutrients or oxygen gives Scots pine an advantage in adverse soil conditions. Compared with many other species (Chapin and Kedrowski 1983, Killingbeck 1996), Scots pine is more proficient at resorbing nitrogen from senescent leaves. In more fertile soil types, pines achieve a higher level of nitrogen in foliage than in poor sites, but the level to which the nitrogen concentration of the senescent needles declines seems to be similar in different habitats. This explains why there was a significant variation in **NRE** between the soil types, but not in **NRP**. The same is valid for inter-specific variation: for example, the **NRE** of understory shrub species growing in an oak-dominated forest differed among species, but **NRP** did not (Killingbeck and Costigan 1988). However, this inverse juxtaposition of **NRE** and **NRP** did not hold in shrub species inhabiting the margins of a desert arroyo (Killingbeck and Whitford 2001). Wright and Westoby (2003), performing a study on 73 Australian evergreen species, found that the nutrient resorption efficiency did not depend on soil nutrient availability, while the resorption proficiency did.

The mean residence time of nitrogen did not statistically differ between pine stands on the different soil types, although the resorption efficiency was higher on the mineral soil. However, variation in **NRE** did not cause equidirectional variation in **MRT**, because the maximum life-span of needles was distributed uniformly between the soil types. Eckstein *et al.* (1999) have argued that nutrient resorption efficiency is more important in explaining variation in **MRT** at the intra-specific level than at the inter-specific level and that the increase in resorption efficiency compensates for a twofold increase in maximum needle longevity (Eckstein *et al.* 1999). In this study, however, the 10% difference in resorption efficiency between the soil types was not big enough to differentiate **MRT** of nitrogen in the foliage of the trees growing in the mineral and in the organogenic soils. The analysis of the pooled data across all study sites indicated that **MRT** of nitrogen in pine foliage was primarily determined by needle longevity (Fig. 3 in **V**). Escudero *et al.* (1992), studying the simulated effects of resorption efficiency on **MRT** at different leaf longevities, proposed that only **NRE** values above 80% would cause **MRT** of nitrogen in short-lived leaves to increase more than the lengthening of longevity of the foliage with smaller resorption efficiency.

When data on both soil types were pooled, trees with higher nitrogen concentration in current-year needles were less proficient in resorbing nitrogen than the trees with low nitrogen concentration (positive correlation between $\mathbf{N_{m2}}$ and $\mathbf{N_{m3}}$; Table 5 in **V**). Thus, trees with higher nitrogen concentration in the current-year foliage lost more nutrients with abscission of old needles. The **NRE** was not related to $\mathbf{N_m}$ of the green needles, which agrees with the results of Chapin and Moilanen (1991) and Escudero *et al.* (1992), but not with those by Nordell and Karlsson (1995). However, a wider range of $\mathbf{N_m}$ values should be covered to draw reliable conclusions about the nutritional control of **NRE** in Scots pine needles.

5.6. Needle trace diameter

Although the present results (**VI**) cannot directly be used for retrospective prediction of needle morphometric characteristics from needle trace diameter ($\mathbf{D_{nt}}$), they do point to some general trends that confirm a need for further studies in this field. The ontogenetic development of the vascular system of leaves and shoots has been rather well studied, in angiosperms in particular (Larson 1975, Larson 1984, Nelson and Dengler 1997, Xia and Steeves 2000, Pizzolato and Sundberg 2002). Remarkably fewer studies have been concerned with gymnosperms (Elliott 1937, Ewers 1982, Boddi *et al.* 2002).

The inter-specific differences in $\mathbf{D_{nt}}$ among the studied species are related to needle length. However, needle traces of Scots pine, a species with the longest needles among the studied species, have certain anatomical differences compared with the other species. In the pine genus, the needles have assembled on short shoots attached to the long shoots, whereas the needles in *Picea* or *Pseudotsuga* species do not form short shoots. Thus, the needle traces of pine species are actually signs of the shoots and include pith that is surrounded by xylem (Kurkela and Jalkanen 1990, Jalkanen 1995). Therefore the needle trace diameter in Scots pine might not be greater as a result of long needles, but because of its anatomical peculiarities. When Scots pine was excluded, the other four species showed a strong correlation (r=0.84, p<0.05) between needle size (needle width × needle length) and $\mathbf{D_{nt}}$. Thus, the inter-specific variation in needle trace diameter is related to needle morphology, and therefore the species with bigger needles are more suitable for NTM studies.

In Scots pine, the needle-trace diameter varied among the growth sites, individual trees, and individual shoots. The variation in $\mathbf{D_{nt}}$ was correlated with needle length, although not so strongly (r= 0.36, p<0.05) as in the case of the inter-specific comparison. Several studies have shown that elevated atmospheric CO_2 affects the morphology and structure of plant leaves by decreasing stomatal density (Pearson *et al.* 1995, Woodward and Bazzaz 1995, Lin *et al.* 2001) and increasing needle thickness and the relative cross-sectional area of phloem (Lin *et al.* 2001). We found a positive correlation (r=0.55, p<0.05) between stomatal density and needle trace diameter. Most probably, the area of xylem in the vascular bundle might give a stronger relationship with stomatal density than $\mathbf{D_{nt}}$, and it could be used for historical analysis of changes in needle morphology by using needle trace method. As such studies have still not been carried out, we can only state that the bigger the needle trace diameter in shoots of Scots pine, the greater the stomatal density, width, length and dry weight of the needles attached to the shoot tend to be.

Our results confirmed that needle traces contain some retrospective information about needle structure, and this information can be obtained by using NTM. Although the strength of the relationships was not enough to make unequivocal predictions, further studies in this field are justified in the light of the findings presented here.

6. CONCLUSIONS

1. Strong correlation between the Estonian and Finnish long-term chronologies of needle retention in Scots pine suggests that regional factors (e.g. yearly fluctuations in weather), rather than local factors (e.g. forestry practice, local sources of air pollution), determine needle retention patterns. There was no unidirectional trend in the chronology of needle retention in 1960–1980s.
2. As trees age, the mean needle longevity in Scots pine increases while needle production, needle shedding, and overall number of needles fluctuate around a mean level for a long period, after which they start to decrease sharply. Older trees (>50 years) have relatively more needles of older age classes compared with younger trees, but, in absolute numbers, they have fewer needles per stems.
3. The maximum longevity of needles does not differ among forest types (*Vaccinium*, pine bog, and dry heath forest types), but the mean needle survivorship has a community-specific pattern, being smaller in stands growing in fertile sites. The variation in needle morphology (needle surface area, **SLA**), mass-based foliar nitrogen content, and nitrogen conservation is also related to forest type. In less fertile sites, the needles are smaller and have higher dry mass–area ratio. In fertile sites, pines achieve a higher nitrogen concentration in foliage than in poor sites, but the level to which the nitrogen concentration of the senescent needles declines (i.e. nitrogen-resorption proficiency) is similar for different habitats.
4. The mean residence time of nitrogen in pine foliage is primarily determined by the longevity of needles, not by nitrogen-resorption efficiency.
5. The diameter of needle traces in shoots of Scots pine is related to stomatal density, width, length, and dry weight of the needles and, in principle, could be used for retrospective prediction of needle morphometric characteristics.

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SUMMARY IN ESTONIAN

Hariliku männi okaste eluea varieerumine, selle seos okaste morfoloogia, lämmastiku taaskasutamise ja puu vanusega

Mitmeaastast lehestikku käsitletakse sageli kui taimede kohastumust kasvuks kõrgenenud stressitasemega keskkonnas. Liigisiseselt on täheldatud lehtede eluea varieerumist liikudes põhja-lõunasuunas või mägedes mööda kõrgusgradienti. Arvatakse, et selle põhjuseks võib olla kasvukoha temperatuurirežiimi muutumine. Ent lehtede eluiga varieerub ühe liigi piires ka märksa väiksemal ruumiskaalal — näiteks võib okaste eluiga samas puistus kasvavatel okaspuudel erineda mitme aasta võrra. Seni on jäänud ebaselgeks, kas selline varieeruvus peegeldab kindlaid seaduspärasusi, mis võiksid tuleneda liigi võimest kohaneda muutuvate keskkonnatingimustega.

Käesolevas doktoritöös on analüüsitud hariliku männi (*Pinus sylvestris* L.) okaste eluiga iseloomustavate tunnuste varieerumist ja selle seoseid okaste morfoloogia ning lämmastiku taaskasutamisega. Tähelepanu on koondatud järgmistele küsimustele:

1. Milline osatähtsus on lokaalsetel ja regionaalsetel teguritel männiokaste eluea pikaajalises varieerumises?
2. Kas okaste eluiga ja dünaamika sõltuvad puu vanusest?
3. Kas okaste eluiga ja okaste morfoloogiat ning lämmastiku taaskasutamist iseloomustavad tunnused on omavahel seotud ning kas nende tunnuste varieerumises ilmneb kasvukohaspetsiifika?
4. Kas lämmastiku keskmine püsivusaeg lehestikus sõltub lämmastiku resorptsiooni efektiivsusest või okaste elueast?
5. Kas okkajälje diameeter on seotud okaste morfoloogiat iseloomustavate tunnustega?

Töös kasutatud materjal on kogutud nelja aasta jooksul 38 proovialalt, mis paiknesid Eesti erinevates piirkondades. Sõltuvalt uuringute iseloomust langetati proovialadel suured puud ning transporditi need edasiseks analüüsimiseks laborisse või võeti üksnes proovioksi. Koostöös Soome Metsainstituudi Rovaniemi uurimisjaamaga kasutati võrdluseks Soomes ja Poolas teostatud uurimistöödel saadud andmeid.

Okaste eluiga ja dünaamikat iseloomustati järgmiste tunnuste abil: okaste elumus (elusate okaste suhe sama võrse okaste algsesse arvu), okaste maksimaalne eluiga ($\mathbf{A_{max}}$, okaste vanuseklasside arv), okaste keskmine elumus (elumuste summa $\mathbf{NR_t}$, jagatud okaste maksimaalse elueaga), okaste keskmine eluiga (**A**), okkaproduktsioon (**P**, okaste arv võrsel selle moodustumise aastal), okaste üldarv (**N**, elusate okaste arv järjestikustel võrsetel), okkavaris (**S**, järjestikustelt võrsetelt varisenud okaste koguarv), okkavarise koefitsient (**S/P**). Okaste morfoloogia iseloomustamiseks mõõdeti okaste pikkus, laius ja paksus

ning määrati okaste kuivmass. Nende tunnuste abil leiti okaste eripind (**SLA**) ja okaste massi suhe nende pindalasse (**LMA**). Lämmastiku taaskasutamist iseloomustati lämmastiku resorptsiooni efektiivsuse (**NRE**) ja lämmastiku keskmise püsivusaja (**MRT**) abil.

Töö tulemused võimaldavad teha järgmisi järeldusi:

1. Tugev korrelatsioon Eesti ja Soome proovialade okaste keskmise eluea pikaajaliste aegridade vahel näitab, et olulisem on olnud regionaalsete (nt. aastased fluktuatsioonid ilmastikuparameetrites) kui lokaalsete (metsamajanduslikud meetmed, lokaalne õhusaaste) tegurite mõju.
2. Puude vananedes suureneb hariliku männi okaste keskmine eluiga, sellal kui okkaproduktsioon, okkavaris ja okaste üldarv püsivad suhteliselt kaua ühel tasemel, misjärel hakkavad järsult vähenema. Üle viiekümne aasta vanused puud omavad suhteliselt rohkem okkaid vanemates vanuseklassides, kuid absoluutarvudes on neil võrse kohta vähem okkaid kui noorematel puudel.
3. Okaste maksimaalne eluiga ei erine erinevatel kasvukohtadel kasvavatel mändidel, küll aga on okaste keskmine elumus väiksem viljakamatel kasvukohtadel. Ka okaste morfoloogias (**SLA, LMA**) ning lämmastikusisalduses ilmneb kasvukohaspetsiifika. Vähem viljakatel kasvukohtadel on männiokkad väiksemad ning nende **LMA** on suurem. Viljakatel kasvukohtadel on nii okaste lämmastikusisaldus kui ka lämmastiku resorptsiooni efektiivsus suuremad kui vaestel kasvukohtadel. Varisenud okaste lämmastikusisaldus on ühesugune kõigis kasvukohtades.
4. Lämmastiku keskmine püsivusaeg okastes on peamiselt määratud okaste maksimaalse elueaga mitte lämmastiku resorptsiooni efektiivsuse poolt.
5. Okkajälgede diameeter on harilikul männil positiivselt korreleeritud õhulõhede tiheduse, okka laiuse, pikkuse ning kuivmassiga. Okkajälje diameetrit saab põhimõtteliselt kasutada männiokaste morfoloogia retrospektiivseks analüüsiks.

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PUBLICATIONS

I

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Needle chronologies on *Pinus sylvestris* in Northern Estonia and
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Needle Chronologies on *Pinus sylvestris* in Northern Estonia and Southern Finland

Margus Pensa and Risto Jalkanen

Pensa, M. & Jalkanen, R. 1999. Needle chronologies on *Pinus sylvestris* in northern Estonia and southern Finland. Silva Fennica 33(3): 171–177.

A needle trace method was used to reveal the chronology of needle retention on Scots pine (*Pinus sylvestris* L.) in southern Finland (two stands) and northern Estonia (two stands). The average annual summer needle retention along the main stem varied from 2.2 to 3.1 in Estonian stands and between 3.4 and 4.2 in Finnish stands during the period 1966–1990. The 23-year-mean needle age was 3.0 and 2.1 years in Finland and Estonia, respectively. In all stands, the mean needle age decreased sharply in 1980s.

Keywords crown thinning, needle trace method, needle age, needle retention, needle loss, shedding
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1 Introduction

In the early 1980s, a severe and rapidly increasing decline of coniferous forests attracted public attention in Central Europe. To obtain a clear picture of the extent and severity of damage, forest health surveys were carried out in many countries. According to the percentage of needle loss and the discoloration of the foliage, the damage category of forests was assessed. However, the methods of damage assessment had been heavily criticised. It had been suggested that knowledge about the natural variation in needle loss were not sufficient to draw border between healthy and damaged trees (Blank 1985). The second problem was the missing data about long-term variation in needle retention. So it was impossible to compare the extent of defoliation with the past thinning of crowns (Blank et al. 1988). Thirdly, the available methods for forest health assessments were highly subjective. There were reports that if the same methods were used by the different researchers on the same trees, the results were often very different (Innes 1993).

In the beginning of the 1990s, a new method for a retrospective assessment of needle reten-

tion on pine trees was developed (Kurkela and Jalkanen 1990). The needle trace method (NTM) allows quantifying the long-term variation in needle retention on a single tree. It has been used for revealing a past needle-cast epidemic (Jalkanen et al. 1994a) and a long-term negative impact of air pollution on needle retention (Jalkanen 1996). Additionally, the NTM has produced the chronology of needle retention in two pine stands in England (Jalkanen et al. 1994b) and in numerous stands in northern and southern Finland (Jalkanen et al. 1995).

The aim of the present study was to obtain the long-term data about needle retention in Scots pine (*Pinus sylvestris* L.) in northern Estonia, and to compare those data with the long-term needle retention of pine trees grown in southern Finland.

2 Material and Methods

Two Scots pine stands were chosen in southern Finland in 1990, and in northern Estonia in 1998. In Finland the stands were located in Tuusula and in Ruotsinpyhtää, in Estonia the locations were in Kose and in Lehtmetsa. In each stand ten pine trees were felled according to guidelines provided by Aalto and Jalkanen (1998). The trees belonged to the main storey not dominated by older trees. They had straight, unbroken stems, and their crowns were regular-shaped (Table 1).

Stands in Ruotsinpyhtää and in Tuusula, in southern Finland, and in Lehtmetsa, in northern Estonia, were naturally seeded; Kose had been seeded by man. The stands in southern Finland had been regularly thinned with the interval of 10 years while Estonian stands had grown without interference during the studied period. The trees from the stands in southern Finland were the same as were previously investigated by Jalkanen et al. (1995).

Prior to the felling of the trees, the compass direction was marked on the east side of each tree. After felling, the compass direction was extended along the whole length of each stem. On every tree, the number of needle sets was assessed in 25 per cent classes for the main stem and also for 3 to 5 branches from the 10th whorl from the top of the tree. Discs from each tree were obtained at breast height for subsequent measurements of radial increment. The parts of the stems above breast height were sectioned into bolts, corresponding to annual shoots, by omitting the branch whorls as instructed by Aalto and Jalkanen (1998). However, the youngest (the most recent 5–9 years) sections of the stems were kept intact while they were transported to laboratory.

Table 1. Basic information of the sampling sites.

Southern Finland

Location	N	E	Altitude, m a.s.l.	Thermal sum*, deg. days	Forest site**	Age, yr	Height, m	d.b.h., cm	Trees no.
Ruotsinpyhtää	60°32'	26°27'	30	1320	sub-dry	45	15.1	16.3	10
Tuusula	60°21'	24°57'	60	1290	sub-dry	53	16.3	16.0	10
Mean			45	1305		49	15.7	16.15	10

Northern Estonia

Location	N	E	Altitude, m a.s.l.	Thermal sum, deg. days	Forest site	Age, yr	Height, m	d.b.h., cm	Trees no.
Lehtmetsa	59°12'	25°35'	60	1650	sub-dry	65	18.7	14.9	10
Kose	59°19'	27°32'	45	1700	sub-dry	40	17.7	14.9	10
Mean			52.5	1675		52.5	18.2	14.9	10

* Treshold value +5 °C.
** *Vaccinum vitis-idaea* was the dominant species in ground vegetation in all stands.

In the laboratory, each annual bolt was planed along the marked east side, tree ring by tree ring, towards the pith of the shoot, and the number of needle traces in the planed surface of the 5–6 innermost tree ring was recorded. Thus, it was assumed that the age of the oldest needles would be no more than six growing seasons. The recordings of the needle trace data were used for calculating annual needle retention, annual needle loss and mean needle age. The needle retention (NR_t) value was calculated as the sum of percentages of needles on each shoot in year t:

$$NR_t = \frac{\sum[x_t,(x-1)_t \ldots (x-n)_t]}{100}$$

where x_t is the percentage of needles on shoot x present in year t, $(x-1)_t$ is the percentage of needles in year t on the shoot initiated the year before shoot x, and so on. Consequently, annual needle loss (ANL_t) was calculated by:

$$ANL_t = (NR_t - NR_{t+1}) + 1$$

where the term 1 represents the new flush of needles in year $(t+1)$. The formula for computing mean age of needles (NA) born in a certain year was following:

$$NA = \sum_{r=1}^{n}\left[(x_r - x_{r+1})\left(r - 1 + \frac{m}{12}\right)\right]$$

where x_r is the relative number of needles x in annual ring r in the bolt, m is the number of months between the birth of new needles and the yellowing of oldest needles (this was taken as three months in present study). Both the planing procedures and calculating procedures followed the instructions compiled by Aalto and Jalkanen (1998). Data were analysed by the NTM program, which was specifically constructed for the needle retention research at the FFRI.

The statistical differences between the Estonian and Finnish study stands were investigated by means of paired t-test at significance level $p = 0.05$. The trendlines describing long-term changes in needle retention with time were estimated by non-linear transformation, multiple regression analysis (Statistica 1998).

3 Results

3.1 Needle Retention

The period during which the data from both countries were comparable was 25 years long, from 1966 to 1990. During that period, annual average needle retention values for summer time varied from just above 3.1 to 2.2 needle sets in Estonia, and between 3.1 and 4.2 in Finland (Fig. 1a). The 25-year-mean of 20 trees in Estonia (mean ± SD = 2.8 ± 0.2) was significantly lower ($p < 0.001$) than in Finland (3.7 ± 0.2). The long-term difference in needle retention within study areas was not significant for both Finland and Estonia (0.1 needle sets between Tuusula and Ruotsinpyhtää, and 0.0 between Lehtmetsa and Kose). As the trees from Finnish study stands had a typical long-term pattern best described by a convex trendline ($R^2 = 0.53$, $p < 0.001$), the trees from Estonian stands were with less obvious concave pattern ($R^2 = 0.09$, $p < 0.05$) (Fig. 2). However, the short-term pattern was quite similar: the peaks up and down happened parallelly in needle retention chronology (Fig. 1a).

3.2 Needle Age

Average standwise needle age varied annually between 2.6 and 3.4 years in Finland, and 1.5 and 2.3 years in Estonia from 1964 to 1986 (Fig. 1b). The 23-year-mean needle age was significantly higher ($p < 0.001$) in Finland (3.0 ± 0.2) than in Estonia (2.1 ± 0.2). The average minimum occurred in Kose in 1980 (1.4 ± 0.3), and in Lehtmetsa in 1981 (1.3 ± 0.5); these values were significantly lower ($p < 0.001$) than the long-term average of mean needle age. In Finnish experimental stands the minima were achieved in 1980 (2.7 ± 0.4 and 2.7 ± 0.6 in Tuusula and in Ruotsinpyhtää, respectively), which differed significantly ($p < 0.05$) from long-term value of mean needle age.

3.3 Needle Shed

The average (within country) amount of annual needle loss varied between 0.6 and 1.5 needle

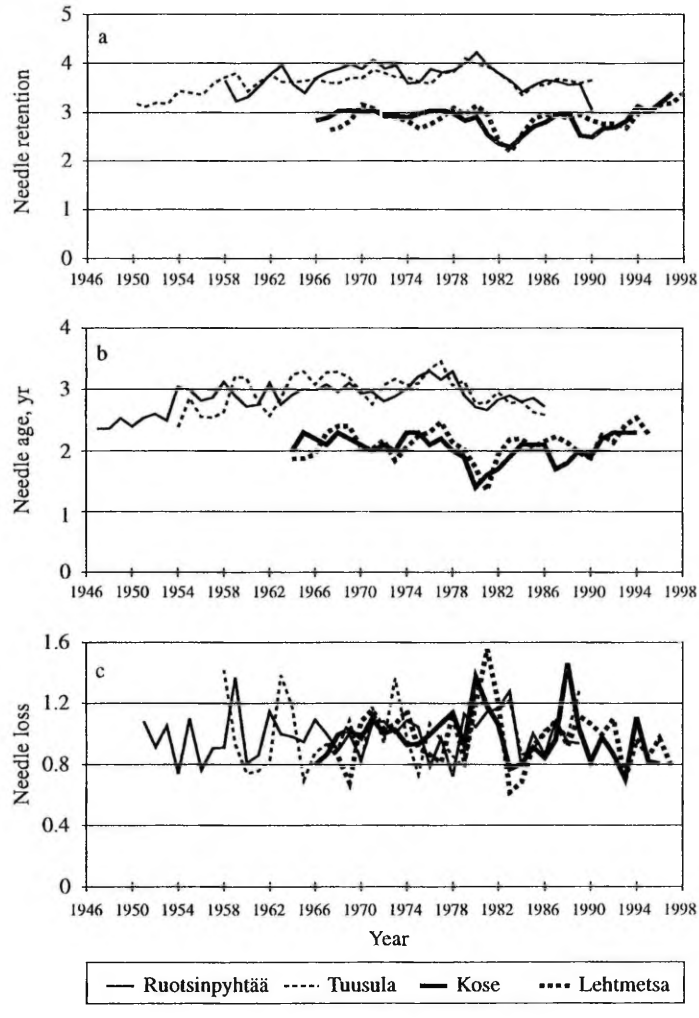

Fig. 1. Needle chronologies on *Pinus sylvestris* in Kose and Lehtmetsa in northern Estonia, and in Tuusula and Ruotsinpyhtää in southern Finland, a) needle retention; b) needle age; c) needle loss.

sets in Estonia, and 0.7 and 1.4 in Finland (Fig. 1c). The pattern of needle loss was quite similar within country. The 23-years (from 1967 to 1989) means of needle loss did not differ significantly between countries ($p = 0.8$). There had occurred a strong shedding in Estonia in 1980–1982 (both stands) when up to 1.5 needle sets was lost. The same happened in Finland in 1980–1983 (Ruotsinpyhtää, 1.3 needle sets) and in 1979–1983 (Tuusula, 1.2 needle sets). Needles were shed younger in Estonia than in Finland (Fig. 3). From 7.3 to 8.4 % of all needles lived only one growing season in Estonia, whereas there were only 0.3 to 1.1 % of needles in this class in Finland. Correspondingly 14.9–15.3 % of the needles lived five years, some (0.5–1.0 %) even

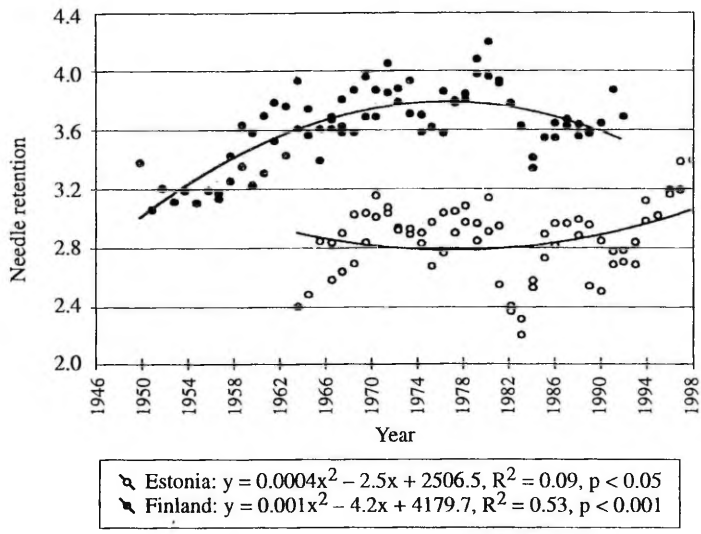

Fig. 2. Long-term changes in needle retention with time.

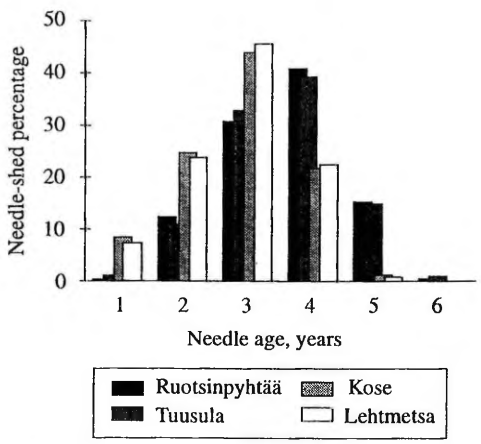

Fig. 3. Distribution of needle-shed percentage according to needle age.

six years in Finland, but only 0.8–1.2 % of the needles lived five years in Estonia (Fig. 3). Medium age for needle shedding was three and four years in Estonia and in Finland, respectively.

4 Discussion

Expectedly, the number of needle sets was greater in southern Finland than in northern Estonia. This is in good agreement with the latitudinal variation in the number of needle sets as shown by Pravdin (1964) in Russia, and by Jalkanen et al. (1995) in Finland. The correlation between the number of needle sets and latitude is related to the latitudinal variation in thermal sums (Jalkanen et al. 1995). The impact of thermal sum on the number of needle sets indicates an important role of climate (summer temperatures) in determining the level of needle longevity. Variation in the mean needle retention among the studied stands was the same in both countries.

Variation in needle retention with time showed two types of temporal variation. The first type of variation was connected to entire period under examination, from about breast height age to the present. In Finnish experimental stands the needle retention increased at first, then it reached its maximum and in the last stage it began to decrease. As have been shown in earlier reports, such pattern of needle retention was found also in southern England (Jalkanen et al. 1994b) and

in northern Finland (Jalkanen et al. 1995). However, in Estonian study stands the trees had opposite pattern – the needle retention decreased at first, then it began to increase. The factors having influence on long-term pattern of needle retention are unknown yet, but the difference between Estonian and Finnish stands allows to suppose that there is not a general way how the needle retention varies over the lifespan of Scots pine. The explanation could rather be sought from the differences in silvicultural procedures. As the stands in Finland were regularly thinned with the approximate interval of 10 years, the stands in Estonia had grown undisturbedly from the very beginning until 1996 in Lehtmetsa and 1997 in Kose. Thus in Finland the trees grew under better light conditions than in Estonia, which might induce the needle retention to increase until the canopy closure of the stands.

Interestingly, the number of needle sets as well as the mean needle age began to increase in both stands in Estonia in 1990s. Due to the absent of data about southern Finland, it was not possible to compare trends of 1990s between Estonia and Finland. Thus it is unclear whether this is a regional or merely a local phenomenon.

The second type of variation included short-term changes in the level of needle retention, which was notable in all experimental stands. To find out the reason for this variation, it is necessary to examine the factors influencing the mean age of needles flushed in a certain year. The mean age of needles varied up and down with 2–7 years intervals, which caused corresponding variation in needle retention and in needle loss (Fig. 1). This short-term variation can not be caused by silvicultural practises because the intervals between repeated peaks are too short, and in Estonia the stands had not been thinned before the mid 1990s. The simultaneity of the pointer years in needle age chronology suggests that the factors causing short-term variation are regional, rather than local. One drastic decrease in needle age occurred at the beginning of 1980s. In all study stands the needles born then lived significantly less as compared with the long-term average life duration of needles (Fig. 1b).

The climate could be one of the factors that have an important role in determining the short-term variation in needle longevity. However, the sharp decrease in mean needle age in the first half of 1980s can hardly be explained only by weather conditions. The decline of mean needle age was prevailing 2–3 successive years but there was not such a steady trend in weather conditions either in Estonia or in Finland at that time. Although, if considering the circumstances for carbon assimilation, the totally different summers – drought in 1976 and extreme rainfall in 1978 and 1979 (and therefore a low level of irradiance) – might cause the rate of net photosynthesis to be dropped. This in its turn might have influenced the state and hardiness of the needles born in those years and soon after. Nevertheless, the decrease of mean needle age in early 1980s observed in this study was nearly simultaneous with the widespread damage of coniferous forests in western Europe, so it is likely that in both cases there was the same reason. In western Europe the forest decline was thought to be a direct consequent of air pollution. The far spreading of air pollutants in combination with climate might also give rise to the decrease in needle longevity in areas under discussion.

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Influence of the alkaline emissions produced by the oil-shale industry on needle retention and needle age of Scots pine

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Abstract. The needle trace method (NTM) was used to reveal retrospectively the needle history of *Pinus sylvestris* L. affected by long-term alkaline deposition caused by the oil-shale industry in Kose, NE Estonia. The Kose site was controlled using sites in Suonenjoki, central Finland, Lehtmetsa in northern Estonia, and Wyszkow in NE Poland, which represented low, medium and high levels of acidic deposition, respectively. Needle retention decreased southwards from 3.2 needle sets in Suonenjoki to 2.9 sets on both of the Estonian sites and 2.4 sets in Wyszkow. Minimum in needle retention was reached in Finland and Estonia in the early 1980s. The parallel development of needle retention and needle age in Kose and Lehtmetsa, on the one hand, and in Suonenjoki and the Estonian sites, on the other hand, from the 1960s to 1990s strongly suggests that alkaline deposition has had no influence on the variation of these needle parameters.

Key words: *Pinus sylvestris*, air pollution, fly-ash, needles, defoliation, crown thinning, NTM.

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Introduction

It is well known that air pollutants affect the foliage and decrease the number of needle sets, i.e. needle retention by decreasing the average age of the needles (or needle longevity). These effects are sometimes toxic, resulting in direct injuries in needles. Often needles are influenced indirectly via physiological processes. Point source emissions from a Cu-Ni smelter combined with high SO_2 levels are a good example of toxic influences reducing needle longevity in the surrounding pine forests (Jalkanen, 1996).

As opposed to vast amounts of emissions, lower levels of depositions are more difficult to recognise in terms of needle retention, despite occurring in the vicinity of a point source emitter. The situation is even more different when the emissions and depositions are alkaline. So far we do not know how needle longevity and needle retention behave in conditions when the main part of the deposition is alkaline.

The aim of this research undertaking was to study the influence of long-term alkaline deposition on the needle longevity of Scots pine (*Pinus sylvestris* L.) in the vicinity of oil-shale industrial activity in NE Estonia. Needle retention and needle age data were produced retrospectively by means of the needle trace method (NTM) (Kurkela, Jalkanen, 1990).

Material and methods

Four stands of Scots pine in the pole stage were chosen as the subject of examination to find out retrospectively their needle history. The stand subjected to alkaline deposition was located at Kose, NE Estonia (59°19'N), seven kilometres from the Ahtme Power Plant using oil shale for energy generation. It was controlled by the Lehtmetsa stand (59°12'N), 130 km west from Kose, northern Estonia. Lehtmetsa was considered to be a non-polluted stand as concerns alkaline deposition. We refer to it as the Estonian control, and at the same time it represented a stand subjected to medium level of acidic deposition. In order to better rule out alkaline influences, these two sites were controlled by a stand in Suonenjoki (62°39'N), central eastern Finland, 400 km north from Kose, representing a site subject to low acidic deposition. A stand with high acidic deposition was obtained from Wyszkow (52°40'N), eastern Poland, which is located 820 km SSW of Kose (Figure 1).

The needle trace method (Kurkela, Jalkanen, 1990; Jalkanen *et al.*, 1994[1]) was used to reveal the needle history of the pine trees. Sampling involved the felling of ten pine trees (eight in Suonenjoki) in 1998–1999, based on the guidelines provided by T. Aalto and R. Jalkanen (1998[1]). The sample trees, which belonged to the dominant storey, had straight undamaged stems, and their crowns were regular in shape. The stands were between 35 years (Wyszkow) and 60 (Lehtmetsa) years old with the average heights of the sample trees being 17.7, 18.7, 13.3 and 17.4 m, and their mean diameters were 14.9, 14.9, 13.3 and 16.2 cm (representing the locations Kose, Lehtmetsa, Suonenjoki and Wyszkow, respectively).

Prior to being felled, the trees were marked on the stem to indicate their east-facing side. After felling, this mark was extended along the whole stem, indicating the side where the needle traces were subsequently observed in the laboratory (Aalto,

Figure 1. Location of the sampling sites

Jalkanen, 1998[1]). Discs were cut from each tree at breast height (b.h.) to facilitate the measuring of radial increment. The stem between b.h. and the top was cut into sections equalling the annual shoots. Prior to this, the lengths of the annual shoots were measured. Each annual-shoot bolt was about 15 cm in length.

In the laboratory, the 5 to 8 innermost tree rings of each bolt were analysed for needle traces by using the arc surface technique of the NTM (Aalto, Jalkanen, 1998[1]), which is more accurate than the plane surface (Jalkanen *et al.*, 2000). The needle trace data were computed based on T. Aalto and R. Jalkanen (1998[2]) to produce needle retention chronologies (Jalkanen *et al.*, 1995, 1998; see also elsewhere in this book). In the following, we mainly concentrate on summer needle retention and needle age. Needle age was calculated as the actual age rather than in terms of growing seasons. The numbers of all intact needles were transformed shoot-specifically to relative values (0.0 = no needles left; 1.0 = full needle set, all needles intact), which then were summed and this resulted in needle retention values (e.g. 2.8 sets). In all the chronologies, a single annual value represents always the mean of at least five trees.

The trees' radial increments were measured by accessing the stem discs in the east and west directions applying an accuracy of 0.01 mm. Arithmetic means of two measurements were used in the analysis. No standardization of any chronology was carried out.

Air pollution levels at Kose

The annual average levels of oil-shale fly-ash and SO_2 in the air in Kose were calculated by using the data base of the air pollution sources and the computer program IMIT, both designed at the Institute of Ecology, Tallinn University of Educational Sciences (Liblik, Kundel, 1993; Liblik *et al.*, 1994). Because of the gaps in the database, the mean annual concentrations of air pollutants were calculated applying intervals of five years from 1960 to 1980, and then for 1990 and 1995.

The annual mean concentrations of oil-shale fly-ash in the air were very high from 1960 to 1975, ranging up to 95 μg m^{-3}. After 1977, when electrostatic filters were installed in Ahtme PP, the emission of fly-ash dropped abruptly to 6.11 μg m^{-3} by 1980. Subsequently, the concentration of fly-ash declined even more due to the economical recession, averaging 5.25 and 2.25 μg m^{-3} in 1990 and 1995, respectively. The short-term maximum concentrations of fly ash ranged from 2278 to 3294 μg m^{-3} in 1960–1975, and from 54 to 202 μg m^{-3} in 1980–1995. On the contrary to this, the annual mean concentration of SO_2 in the air was more stable during the period under discussion: it varied between 2.47 and 10.3 μg m^{-3}. The short-term maximum concentrations of SO_2 ranged from 76 to 314 μg m^{-3}.

Results

Tree growth

Despite the different silvicultural histories of the experimental stands, the non-standardized tree ring chronologies from Suonenjoki and Wyszkow correlated significantly with that of Kose (r=0.41 and 0.68, respectively). Despite a distance of over 1,200 km between the sites, the radial growths of the stands in Suonenjoki and Wyszkow also correlated significantly (r=0.60, $p<0.001$). This correlation was at its highest between the Estonian sites (r=0.88 for radial growth).

Although the Estonian sites correlated with each other with high statistical

significance (r=0.80) as concerns height increment, there was no correlation, or the dependence was negative, between Kose and the control sites in Finland and Poland. This would appear to indicate differences in forest management and stand density rather than in pollution.

Needle retention

The stand-specific means for needle retention in Kose, Lehtmetsa, Suonenjoki and Wyszkow were 2.9, 2.9, 3.2, and 2.4 needle sets, respectively. The tree-specific means varied between 2.2 and 3.3, 2.5 and 3.2, 2.7 and 4.1, and 2.2 and 2.5.

Needle retention showed a minimum in the early 1980s on the Estonian and Finnish sites. Medium frequency variation seemed to be stronger in Suonenjoki than in Estonia, and the long-term patterns of the Estonian and Finnish sites differed especially in the early 1980s, the Suonenjoki site showing a less pronounced peak downwards as compared to the Estonian sites. There were hardly any differences between Kose and Lehtmetsa, their needle retention patterns being highly synchronous (r=0.65, p<0.001). The long-term pattern first decreased from the 1960s to the early 1980s, and then started to increase towards the 1990s (Figure 2a). Suonenjoki and the Estonian sites correlated highly significantly (r=0.51–0.56). On the contrary to this, Wyszkow, the Polish site, did not correlate at all with any of the three more northern sites. Also its needle retention pattern clearly deviated from those of the more northern sites.

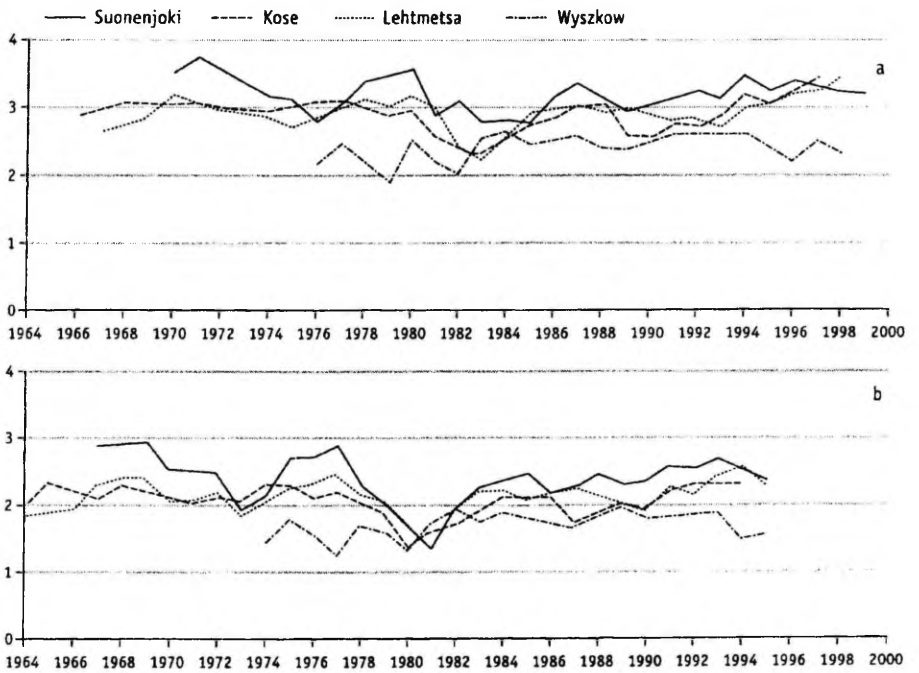

Figure 2. Time series for a) needle retention and b) needle age of *Pinus sylvestris* at Suonenjoki in Finland; Kose, and Lehtmetsa in Estonia; and Wyszkow in Poland

Needle age

The average needle age of Scots pine during the past 23 to 36 years prior to sampling was 1.7 years in Poland, 2.1 years on both of the Estonian sites, and 2.4 years in Finland. The annual stand-specific means varied from 1.4 to 2.3 years in Kose, 1.3 to 2.5 years in Lehtmetsa, 1.9 to 2.9 years in Suonenjoki, and 1.2 to 2.0 years in Wyszkow. The tree-specific means were between 1.5 and 2.5, 1.7 and 2.7, 1.9 and 3.3, and 1.5 and 1.8 years at Kose, Lehtmetsa, Suonenjoki and Wyszkow, respectively.

The needle age pattern was synchronous in the Estonian and Finnish sites, showing first a decreasing trend from the 1960s to the early 1980s, when the lowest values were found to occur in all three areas. After the minimum value, the needle age increased into the mid-1990s. The recent increase in the1990s was very marked on the Estonian sites as compared to Suonenjoki, where it showed a slight downward trend. Annual variation was at its greatest in Suonenjoki, which showed best of all the medium-frequency signal with a 7–8-year cycle. Synchronosity was supported by highly significant correlation coefficients between the three sites (r=0.61–0.69) (Figure 2b). In Wyszkow, the needle age pattern was different, although it had one of its peak downs in 1980 and an increasing trend since 1980. However, correlation analysis with a series consisting of only 21 years gave no significant differences between Wyszkow and any of the other three sites.

Discussion

Comparisons of raw radial growth proxies showed clearly that the climatic conditions of Scots pine in Lehtmetsa, northern Estonia, Suonenjoki, central Finland, and Wyszkow, NE Poland vary in the way that they do in Kose. This suggests that at least radial growth seems not to have been influenced by the very high alkaline deposition in Kose. Contrarily to this, the differences in height increment were more evident. This indicates, however, differences in stand density and silvicultural practices rather than in the quality of deposition.

As Lehtmetsa, located 130 km west from Kose and receiving hardly any alkaline deposition, had very similar needle retention and needle age values and long-term patterns as Kose, alkaline deposition cannot be the main influencing factor in the annual variation in needle retention and needle age. This is supported also by the Suonenjoki control site in central Finland, where deposition is acidic but the long-term needle patterns were similar to the situation in Estonia. The needle retention pattern in Suonenjoki was comparable with the earlier findings in Finland (Jalkanen *et al.*, 1995; see also Jalkanen *et al.*, 1994[2]) and Estonia (Pensa, Jalkanen, 1999). The Wyszkow site in NE Poland is known to have been subjected to high SO_2 and NO_x depositions. This may partly explain why the needle retention pattern in Poland differs from those of the other examined sites.

Because of the gaps in the data on pollutant concentrations, it was not possible to find a definite correlation between pollution levels, needle retention and tree growth. However, the variation in growth and needle retention on part of the trees growing in Kose over time can hardly be explained by changes in the air pollution levels.

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Age-dependent Changes in Needle-fascicle Dynamics of *Pinus sylvestris* (L.)

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Age-dependent dynamics of needle fascicles on the main stem of Scots pine trees in Estonia and Finland were investigated by the needle trace method. Mean fascicle longevity increased with tree age. Fascicle pool size, fascicle production and shedding were not related to tree age for trees less than 50 yrs old. Thereafter, the mean values of these characteristics declined linearly. Thus, younger trees had more fascicles attached to the stem, but fascicle survival in the third and fourth age classes tended to be greater in older trees. A short-term pattern of variation in dynamics was seen: higher values in the rates of fascicle shedding and production, and in the quotient of fascicle loss, in any one year were regularly followed by lower values in the following year. *Key words: needle trace method, Scots pine, senescence.*

INTRODUCTION

The extent to which plants vary in their leaf lifespan has been investigated from several different aspects. Increases in leaf longevity among species have been explained as an adaptation to low nutrient availability (Monk 1966, Chapin 1980, Coley et al. 1985) or, in general, to conditions in which growth is suppressed by any environmental stress factor (Grime 1977, Chabot & Hicks 1982, Reich et al. 1992).

Compared with the genetically determined interspecific variation, the intraspecific variation in leaf lifespan is less pronounced (Eckstein et al. 1999). Differences in leaf longevity within species are observable along latitudinal or altitudinal gradients (Ewers & Schmid 1981, Jalkanen et al. 1995, Reich et al. 1996), which can be explained by climatic variations (Weidman 1939, Jalkanen et al. 1995). Besides variation on a large spatial scale, variation also occurs on a smaller scale, since individuals of the same species in adjacent habitats may be subjected to different levels of nutrient, water (Aerts 1989) or light availability (Nilsen et al. 1987, Seiwa 1999*a*, 1999*b*). Variation in light conditions within the canopy of individual trees may lead to increasing leaf lifespans from the upper to the lower parts of the canopy (Niinemets 1997).

Because plants may experience a wide range of environmental conditions during their lives, they also exhibit temporal variation in leaf lifespan in response to changing conditions. For example, over a 23 yr period the mean fascicle longevity of *Pinus sylvestris* L. varied between 2.6 and 3.4 yrs in southern Finland, and from 1.5 to 2.3 yrs in northern Estonia (Pensa & Jalkanen 1999). In *Ulmus davidiana* var. *japonica* Nikai and in *Acer mono* Maxim., the leaf phenology of seedlings differed from that of the mature trees, which was explained as an acclimation of seedlings to a lower level of irradiance (Seiwa 1999*a*, 1999*b*). However, independently of light acquisition, an increase in tree height or age is correlated with a decrease in carbon assimilation rates and stomatal conductance, caused by a decline in vascular hydraulic conductance (Yoder et al. 1994, Hubbard et al. 1999). The strong relationship between carbon assimilation rate and leaf age has been shown by several interspecific and intraspecific comparisons (Chabot & Hicks 1982, Reich et al. 1992, 1995, 1997). Therefore, leaf longevity should increase, and the corresponding changes in leaf population dynamics should occur, independently of light acquisition during the development of individual trees. If such an age-dependent pattern in leaf life expectancy exists, it should be considered in forest health surveys where

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defoliation is used as an important indicator of forest condition (Innes 1993).

In this paper it was hypothesized that mean fascicle longevity and fascicle dynamics in the top of the crown of Scots pine (*P. sylvestris* L.), a light-demanding species at both the juvenile and the adult stage, depend on tree age. To test the hypothesis, this study investigated fascicle longevity, survival, production, shedding and fascicle pool size on the main shoots (stems) of Scots pine trees of different age, by means of the needle trace method (NTM).

MATERIALS AND METHODS

Study sites and plant material

Four Scots pine stands were chosen for sampling. Two stands were situated at Tuusula (60°21′ N and 24°57′ E) and Ruotsinpyhtää (60°32′ N and 26°27′ E), southern Finland, and the others at Kose (59°19′ N and 27°32′ E) and Lehtmetsa (59°12′ N and 25°35′ E), northern Estonia. Ten trees were sampled at Kose, eight at Lehtmetsa, seven at Tuusula and six at Ruotsinpyhtää, according to the guidelines provided by Aalto & Jalkanen (1998). The trees belonged to the main storey, and were not dominated by older trees. They had straight, unbroken stems and regular-shaped crowns (Table 1).

The stands at Ruotsinpyhtää, Tuusula and Lehtmetsa were naturally seeded, whereas the stand at Kose had been planted. The stands in southern Finland had been regularly thinned at an interval of 10 yrs, while the stands at the Estonian locations had grown without human interference until 1990. The vegetation type was *Vaccinium vitis-idaea* in all stands.

Before the sample trees were felled, the compass direction was marked on the eastern side of the stems. After felling, the marks were extended along the whole length of the stems. Discs from each tree were taken at breast height (1.3 m) for assessment of tree age. The parts of the stem above breast height were sectioned into bolts corresponding to annual increments, by omitting the branch whorls, as instructed by Aalto & Jalkanen (1998). The youngest sections (the most recent 5–9 yrs) of the stems were kept intact for transport to the laboratory.

Estimation of fascicle demography

Needle-fascicle dynamics were studied by means of the NTM (Kurkela & Jalkanen 1990), based on the assumptions that needle fascicles are produced uniformly around the stem, and that fascicle mortality is uniformly distributed without respect to compass direction. Recent tests of NTM assumptions in pines have confirmed a strong correlation between the NTM estimates and actual data obtained by counting living needle fascicles on the same shoots (Jalkanen et al. 2000; M. Pensa, unpubl.). Moreover, the strong correlation between fascicle retention on the main stem and on branches ($r = 0.78$) indicates that fascicle dynamics on the stem can be used for characterizing that on the branches (Jalkanen et al. 1995). For a detailed description of the NTM and the computational technique, see the instructions compiled by Aalto & Jalkanen (1998).

In the laboratory, the calendar year of shoot formation was marked on each annual bolt. Angles of 40–180°, depending on the thickness of the rings, were marked at the ends of the bolts (Aalto & Jalkanen 1998). The bolts were planed along the eastern side, ring by ring, towards the pith. The legs of the angles at the opposite of each bolt were joined by lines, and the number of fascicle traces within the rectangle formed at the planed surface of the five or six innermost rings was recorded. The survival of fascicles on a shoot was estimated by dividing the number of traces in a particular ring of the shoot by

Table 1. *Main information on the sample trees*

Location	Thermal sum [a] (degree days)	Soil type	Mean age (yrs)	Height (m)	dbh (cm)	Years of shoot formation at dbh
Kose (E)	1700	Loamy sand	40	17.7	14.9	1960–1966
Lehtmetsa (E)	1650	Loamy sand	65	18.7	14.9	1935–1942
Tuusula (F)	1290	Sandy	53	16.3	16.0	1944–1953
Ruotsinpyhtää (F)	1320	Moraine	45	15.1	16.3	1957–1962

[a] Threshold value +5°C.
E: Estonia; F: Finland; dbh: diameter at breast height.

that in the innermost ring. Such a definition of survival is analogous to that used by Whitney (1982), Nilsen et al. (1987). Proceeding from the survival, the mean longevity of fascicles (A, yrs) attached to the particular shoot was calculated as:

$$A = \sum_{r=1}^{i} (x_r - x_{r+1})\left(r - 1 + \frac{m}{12}\right) \quad (1)$$

where x_r is the survival of fascicles on the bolt in the year corresponding to the formation of the ring r, and m is the number of months between the birth of new fascicles and the yellowing of the oldest needles (this was taken as 3 months in this study).

For computing fascicle production (P), i.e. the number of fascicles attached to the shoot in the year of shoot formation, the following equation was used:

$$P = \left(\frac{n \cdot 360^{\circ}}{l_b \cdot a}\right) \cdot l_s \quad (2)$$

where n is the number of fascicle traces in the innermost ring, l_b is the length of the corresponding bolt, l_s is the length of the corresponding shoot, and a is the mean of the angles drawn at the ends of the bolt.

The number of fascicles on a shoot, in any year after the shoot formation, was obtained by multiplying the fascicle production of the shoot by fascicle survival. The overall number of fascicles (N) attached to the stem of the tree in a particular year could then be estimated as:

$$N = \sum_{i=1}^{n} f_i \quad (3)$$

where f_i is the number of fascicles on the ith shoot of the stem.

Shedding of needle fascicles (S), i.e. the number of fascicles shed annually from the main stem of the tree, was calculated as:

$$S = (N_t - N_{t+1}) + P_{t+1} \quad (4)$$

where index t is the year number.

Finally, the quotient of fascicle loss was determined by dividing fascicle shedding by fascicle production in the same year (Nebel & Matile 1992). Mean fascicle longevity was estimated on trees sampled at both the Finnish and Estonian study sites, and fascicle survival, production, shedding, overall number and quotient of fascicle loss only at the Estonian study sites.

Data analysis

After the indices of fascicle demography had been computed, the data were arrayed according to tree age at breast height. It was arbitrarily taken that trees 1.3 m tall were 1 yr old. For each age the means of the variables were calculated and those ages represented by fewer than five trees were discarded. In the subsequent analysis, linear regression was applied to describe long-term changes in the mean values of the characteristics. To detect any short-term regularity, a 1 yr lag time was applied to the data and the partial autocorrelation coefficients for the transformed data were determined. The computer package Statistica for Windows 5.0 (StatSoft, Inc.; Statistica 1998) was used for statistical analysis. Statistical significance was computed at the 0.05 probability level.

RESULTS

Mean fascicle longevity versus tree age

Mean fascicle longevity increased slightly with tree age, although it was very variable from tree to tree. For the Finnish sample trees, the average level of fascicle longevity increased significantly (mean slope $\pm$ SE: 0.005 ± 0.002, $p = 0.02$) from 2.73 ± 0.05 yrs ($p < 0.001$) at the age of 1 yr, to almost 3 yrs at the age of 38 yr (Fig. 1). The increasing trend for Estonian trees (slope 0.002 ± 0.001) was not statistically significant ($p = 0.11$). Comparison of different age classes revealed that survival of the current and previous 2 years' fascicles did not change, while survival in the fourth age class increased significantly (slope 0.19 ± 0.044, $p < 0.001$). Moreover, the older the trees, the more frequently even 4-yr-old needles were present on shoots (Fig. 2A).

Fascicle production

The mean fascicle production fluctuated around a mean level of 240 ± 3.7 ($p < 0.001$) from age 1 to 51

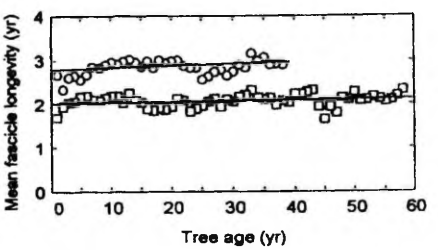

Fig. 1. Mean fascicle longevity on main stem versus tree age. ○: Finnish sample trees ($r^2 = 0.13$); □: Estonian sample trees ($r^2 = 0.05$).

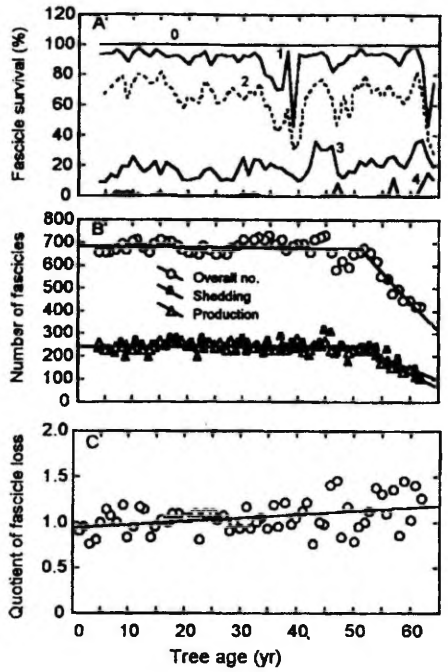

Fig. 2. (A) Survival of fascicles on main stem at different age classes (indicated by Arabic numerals) versus tree age. (B) Dependence of overall fascicle number ($r^2 = 0.83$), shedding ($r^2 = 0.44$) and production ($r^2 = 0.88$) on tree age. (C) Effect of tree age on fascicle loss quotient ($r^2 = 0.16$).

yrs (Fig. 2B), after which it declined sharply (slope -13 ± 1.2, $p < 0.001$) to 100 fascicles by the age of 62 yrs. The partial autocorrelation analysis of the transformed data showed a significant negative correlation ($r = -0.37$, $p < 0.05$), which suggests that years with a high level of fascicle production were followed by years with remarkably lower production.

Overall number of fascicles

The mean overall number of fascicles at the stem was approximately 684 ± 10.6 ($p < 0.001$) for trees aged 4–51 yrs. This was followed by a sharp decrease (slope -26 ± 2.7, $p < 0.001$) in fascicle number (Fig. 2B). The partial autocorrelation of the time-lagged data was insignificant, suggesting that the overall number of fascicles did not alternate regularly in the short term.

Fascicle shedding

Mean fascicle shedding fluctuated around the same level (240 ± 9.3, $p < 0.001$) as fascicle production, but the dispersion of the data was greater (Fig. 2B). The breakpoint occurred at the age of 50 yrs, after which shedding began to decline sharply (slope -10 ± 2.4, $p < 0.001$). Like fascicle production, the transformed data of fascicle shedding showed a regular alternation between high and low levels from year to year ($r = -0.58$, $p < 0.05$).

Quotient of fascicle loss versus tree age

The mean quotient of fascicle loss tended to increase slightly as the trees became older (slope 0.004 ± 0.0011, $p = 0.01$) (Fig. 2C). When fascicle loss was transformed by applying a 1 yr time lag, a significant short-term pattern was revealed: greater quotients were followed by smaller quotients ($r = -0.65$, $p < 0.05$).

DISCUSSION

Recently, Seiwa (1999*a*, *b*) discussed that the differences in leaf longevity between seedlings and mature trees in *A. mono* Maxim. and *U. davidiana* var. *japonica* Nikai may be partly due to ontogenic changes. In the present study a shift was observed in the fascicle longevity on stems of *P. sylvestris* (L.) trees that was age dependent, rather than a consequence of changing environmental conditions. Although it was very variable from tree to tree, the average level of fascicle longevity and the survival of older fascicles tended to increase with tree age. One possible reason for this trend may be the influence of changing water supply on the needles of older or taller trees. Since the height of the sample trees extended from 1.3 m up to almost 18 m (Table 1), there should have been a remarkable difference in needle water supply across the height range of the sample, given that water potential declines by 0.01 MPa m^{-1} owing to the influence of gravity (Nobel 1991). In fact, shoot water potential may decrease even more rapidly, especially in conifers, because of the growing frictional resistance of the xylem to water flow, which is proportional to the length of the transport pathway (Tyree & Ewers 1991). Hydraulic conductance in Scots pine has been reported to be greatest at an age of 15–20 yrs, and to decrease in older trees (Mencuccini & Grace 1996). Annual biomass growth per unit leaf area (growth efficiency) declined with tree age, whereas above-ground sap-

wood volume per unit leaf area, which is related to maintenance respiration costs, increased steadily. Mencuccini and Grace (1996) suggested that increased hydraulic resistance and maintenance respiration costs might be the main causes of reduced carbon gain in mature and old pine trees. Recently, Magnani et al. (2000) proposed a model to explain an age-related decline in pine-stand productivity. According to the model, resource allocation is predicted to shift from foliage to transport tissues, most notably to fine roots, with increasing tree age. Both higher respiration and fine root turnover would result in a decline in net primary productivity. Thus, the increase in needle longevity and survival in older trees may reflect a more economical use of resources under conditions of diminishing net assimilation rates.

The decline in fascicle production observed in trees older than 50 yrs may also be an example of the negative influence of increased hydraulic resistance on tree growth. At the same time, fascicle shedding also declined. In young trees the mean rates of fascicle production and shedding did not differ, but above an age of 50 yrs the decrease in loss was slower than that in production ($p < 0.01$). Thus, the quotient of fascicle shedding of trees older than 50 yrs tended to be > 1.0, indicating that the number of fascicles shed in autumn exceeded the number of needles produced in the previous spring. Therefore, the overall number of fascicles decreased more rapidly than one could expect from the slope of fascicle production. Owing to the increase in fascicle longevity and the decrease in fascicle production, the older trees had relatively more fascicles of the third and fourth age classes compared with younger trees but, in absolute numbers, they had fewer fascicles on the stem altogether.

Mean production, shedding and quotient of fascicle loss exhibited regular short-term fluctuation. Large values of these characteristics were followed by smaller ones in the next growing season. Such regularity is difficult to explain; one explanation might be analogous to the fluctuations in any physiological parameter or population density around an equilibrium value, due to the homeostatic mechanisms which operate in biological systems. The degree of shading along the branches has been shown to determine the amount of foliage in the crown of *Pinus contorta* ssp. *latifolia* (Engelm.) (Schoettle & Smith 1991). The concept of an ecological light compensation point (Schoettle & Fahey 1994) recognizes that leaves have a critical light requirement below which they cannot maintain a positive carbon balance and will be lost. As the tree produces more fascicles in one spring, the degree of shading may increase. This may lead to the negative net carbon balance of older foliage, which brings about both increased fascicle mortality in autumn and decreased fascicle production in the following spring. Thus, the tree can maintain the overall number of fascicles at a more or less constant level, which is optimal for carbon assimilation in the given circumstances.

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IV

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Needle longevity of Scots pine in relation to foliar nitrogen content,
specific leaf area, and shoot growth in different forest types.
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Needle longevity of Scots pine in relation to foliar nitrogen content, specific leaf area, and shoot growth in different forest types

Margus Pensa and Arne Sellin

Abstract: The aim of the study was to examine whether the variation in needle longevity of Scots pine (*Pinus sylvestris* L.) is of a community-specific nature. Altogether 300 trees were examined in *Vaccinium*, dry heath, and pine bog forests in Estonia. Mean number of needle age-classes was 4.1 for all forest types, while mean survivorship of needles averaged 70% in *Vaccinium* forests and 80% in the others ($p < 0.01$). Mean mass-based nitrogen content (N_m) of the needles was the highest in *Vaccinium* forests (11.8 mg·g^{-1}, $p < 0.05$) and lower in dry heath (10.4 mg·g^{-1}) and pine bog (9.8 mg·g^{-1}) forests. Specific leaf area (SLA) did not differ between *Vaccinium* (6.3 mm^2·mg^{-1}) and dry heath (6.0 mm^2·mg^{-1}) forests but was lower in pine bog forests (5.5 mm^2·mg^{-1}, $p < 0.05$). Shoot length increment was the smallest in pine bog forests (45.1 mm·year^{-1}, $p < 0.05$) and did not differ between *Vaccinium* (92.2 mm·year^{-1}) and dry heath (95.2 mm·year^{-1}) forests. Our findings confirm that the small-scale variation in needle longevity of Scots pine has a community-specific pattern and is in accordance with the trends in N_m and SLA.

Résumé : Le but de l'étude consistait à examiner si la longévité des aiguilles chez le pin sylvestre (*Pinus sylvestris* L.) est inhérente à chaque communauté. Trois cents arbres ont été examinés en Estonie dans des forêts à *Vaccinium*, des forêts sèches à bruyère et des pinèdes tourbeuses. Le nombre moyen de classes d'âge des aiguilles était de 4,1 pour tous les types forestiers alors que le taux de survie moyen des aiguilles était de 70% dans les forêts à *Vaccinium* et de 80% dans les autres types ($p < 0,01$). Le contenu moyen en azote sur la base de la masse (N_m) des aiguilles était le plus élevé dans les forêts à *Vaccinium* (11,8 mg·g^{-1}, $p < 0,05$) et plus faible dans les forêts sèches à bruyère (10,4 mg·g^{-1}) et les pinèdes tourbeuses (9,8 mg·g^{-1}). La surface foliaire spécifique (SFS) ne variait pas entre les forêts à *Vaccinium* (6,3 mm^2·mg^{-1}) et les forêts sèches à bruyère (6,0 mm^2·mg^{-1}) mais était plus faible dans les pinèdes tourbeuses (5,5 mm^2·g^{-1}, $p < 0.05$). La croissance en longueur de la pousse terminale était la plus faible dans les pinèdes tourbeuses (45,1 mm·an^{-1}, $p < 0,05$) et était comparable dans les forêts à *Vaccinium* (92,2 mm·an^{-1}) et les forêts sèches à bruyère (95,2 mm·an^{-1}). Nos résultats confirment que la variation à petite échelle de la longévité des aiguilles du pin sylvestre suit un patron spécifique aux communautés et qu'elle suit les tendances de la N_m et de la SFS.

[Traduit par la Rédaction]

Introduction

Intraspecific changes in the leaf longevity of evergreen conifers have frequently been observed along latitudinal or altitudinal gradients (Weidman 1939; Ewers and Schmid 1981; Schoettle 1990; Nebel and Matile 1992; Jalkanen et al. 1995). It has been supposed that such variation reflects the response of the trees to low resource availability or high abiotic stress level, and covaries with trees' growth rate and photosynthetic capacity (Reich et al. 1992, 1999). In less favourable environments it might be a successful strategy to increase the time that nutrients are resident in plants, and this can be achieved in part by increasing the life-span of the foliage (Eckstein et al. 1999).

Some coniferous species may demonstrate rather drastic differences in leaf longevity within their areas of distribution. For example, the needle retention in black spruce (*Picea mariana* (Mill.) BSP) has been reported to vary from 5 to 7 years in southerly reaches of the boreal forest in Quebec, to 13 years in central Alaska, and up to 30 years under subarctic conditions (review by Lamhamedi and Bernier 1994). The intraspecific differences in leaf longevity among conifer populations at different latitudes and elevations appear rather to be phenotypic acclimation than genotypic adaptation (Reich et al. 1996*a*), but the nature of the factors to which conifers have acclimated is still not unequivocally clear. Nevertheless, Weidman (1939) and Jalkanen et al. (1995) have reported that the variation in needle longevity of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) and Scots pine (*Pinus sylvestris* L.) was more closely related to variation in temperature regime than to site elevation or latitude per se.

Besides the variation across climatic zones (along altitudinal and longitudinal gradients), leaf longevity may also vary within a particular climatic zone, if individuals of

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Table 1. Basic characteristics of the three forest types (Cajander 1926; Masing 1996; Paal 1997).

Forest type	Soil type	Soil pH_{KCl}	Total N, %	$Height_{100}$, m	Depth to water table, m
Vaccinium forests	Gleyic Podzols, Umbric Gleysols	2.5–5.5	1.7	25	>2
Dry heath forests	Humic Podzols	3.0–5.0	1.5	14	>3
Pine bog forests	Dystric Histosols	2.6–4.0	0.8	12	>0.20

the same species in adjacent habitats are subjected to different levels of resources. The longevity of leaves in *Rhododendron maximum* L. (Nilsen et al. 1987) and *Acer mono* Maxim. (Seiwa 1999) increased as the light availability decreased. Field experiments on two evergreen ericaceous shrub species indicated that leaf longevity might decrease in response to fertilization (Aerts 1989). Fertilization of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) and grand fir (*Abies grandis* (Dougl.) Lindl.) decreased needle longevity by 26 and 27%, respectively (Blaster and Marshall 2000). Similar intraspecific trends in leaf longevity have been revealed along natural gradients of nutrient and water availability (Jonasson 1989; Nordell and Karlsson 1995; Enoki and Kawaguchi 1999; Kloeppel et al. 2000).

Within an area with uniform climatic conditions, the distribution of soil resources determines the specific pattern of plant communities. The species that are able to form stands across a wide range of habitats should demonstrate a community-specific plasticity both in physiological and morphological traits. The data published recently (Niinemets et al. 2001) for Scots pine indicated that foliar morphological plasticity in response to light availability within a canopy depends on soil fertility. As regards nutrient availability, there are more data on intraspecific variability in foliar morphology but still little evidence of variation in leaf longevity among different plant communities, although the phenomenon has been well documented in general.

In this paper we test the hypothesis that needle longevity of Scots pine, a species with a broad range of tolerance in respect to soil fertility and water availability, has a community-specific pattern. Trees forming plant communities in habitats with low resource availability should have greater needle longevity compared with trees in communities in fertile habitats. Since leaf longevity is related to specific leaf area (SLA), mass-based leaf nitrogen concentration (N_m), and tree growth rates, we examined whether these traits follow similar community-specific pattern as needle longevity. Lower N_m, smaller SLA, and slower growth rate should accompany greater needle longevity of Scots pines growing at nutrient-poor sites. A detailed understanding of the factors that cause variation in leaf longevity of coniferous species is vital to our ability to distinguish natural variability from the consequences of human impact (e.g., air pollution, soil acidification, etc.) and is, therefore, a crucial point for forest health surveys (Innes 1993).

Materials and methods

Study area and sample sites

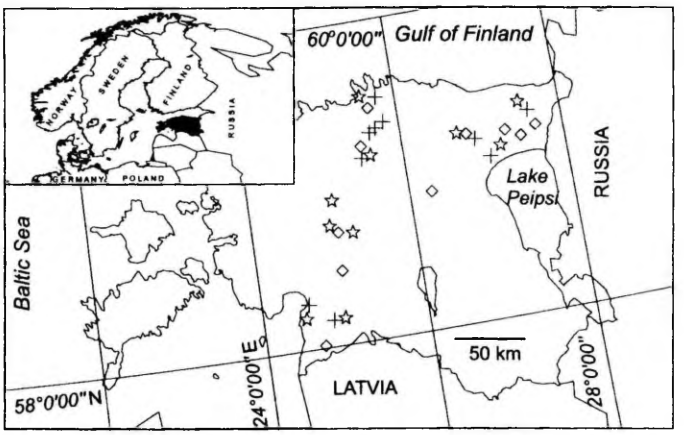
The study was conducted in Estonia, where climatic conditions favour the prevalence of boreal mixed forests. Mean annual temperature in Estonia ranges from 6.0°C in the western part to 4.0°C in the eastern part. Annual precipitation fluctuates between 500 and 700 mm, being greater in southeastern Estonia because of its relatively higher relief (maximum elevation in Estonia is 318 m a.s.l.).

From July to August 2000, we estimated needle longevity in 30 Scots pine stands belonging to three different forest types classified by Masing (1996) as follows: *Vaccinium*, dry heath, and pine bog types. The forest types have been differentiated by a complex of indices related directly or indirectly to soil fertility and water availability (Table 1). The corresponding forest types according to the Cajander's (1926) classification, widely usable in the Nordic countries, are *Vaccinium*, *Calluna*, and pine peat moor types, respectively. We selected 10 study sites in each forest type, 30 sites altogether. All studied stands were situated within narrow latitudinal and altitudinal zones, which eliminated the possibility that differences in needle longevity and related traits might be caused by climatic factors. The dry heath forests are typically spread on very dry and nutrient-poor sands where the depth of the water table is more than 3 m. The dominant species in the ground vegetation are *Calluna vulgaris* (L.) Hull, *Cladina* spp., and *Empetrum nigrum* L. The *Vaccinium* forests grow on sands and sandy loams that are more fertile and have a greater water-holding capacity than the soils of dry heath forests. *Vaccinium vitis-idaea* L. and *Vaccinium myrtillus* L. are the dominant species in the ground vegetation of this forest type. The pine bog forests grow on deep, nutrient-poor, and water-saturated peat; the dominant species in the ground vegetation are *Sphagnum* mosses. The basic characteristics of the forest types are given in Table 1. When carrying out the field observations, we discriminated among the forest types according to the ground vegetation.

Sampling of trees

A total of 300 Scots pine trees were sampled at 30 sites (10 trees per site) between 58°00′N and 59°30′N (Fig. 1). The height of the sample trees varied between 1.5 and 2.5 m. The altitude of the sites ranged from 10 to 110 m a.s.l. At all sites the branches (one branch per tree) were cut from the middle of the eastern side of the sample tree's crown. When sampling the branches, we selected only those that had no needles on at least the two oldest shoot increments. For characterizing maximum needle longevity, we counted the number of needle age-classes on the main axis of each branch. The survivorship of needles in each needle age-class was estimated according to Whitney (1982), Nilsen et al. (1987), and Aerts (1989) by dividing the number of living needles by the sum of the numbers of needle scars and living needles. By dividing the sum of needle survivorships by the number of needle age-classes, we calculated the mean

Fig. 1. Location of the sampling sites; (+) dry heath forests, (☆) *Vaccinium* forests, and (◇) pine bog forests. The inset map shows the location of Estonia (the solid area).



survivorship of needles. This parameter is not related to any certain needle age-class but reflects the mean level of survivorship and is independent of the actual number of needles. Hence, its use is justified to compare needle survivorship among habitats where needle production is very different, while maximum needle longevity is similar. To characterize the growth rate of the trees in different forest types we measured the length of the 1-year-old shoot of the main axis in all sample branches.

Foliar nitrogen content and specific leaf area

For analysing mass-based nitrogen content (N_m, $mg \cdot g^{-1}$ of dry mass) and estimating specific leaf area (SLA, $mm^2 \cdot mg^{-1}$), we collected a total of 825 one-year-old needles. The needles were pooled by the forest types to randomize the sampling procedure. Random subsamples consisting of 35 needles, 5 replications per forest type, were taken from the pooled material, and the N_m was assessed according to Kjeldhal's method at Virumaa Laboratory of Environmental Studies (Jõhvi, Estonia). SLA, expressed on a total surface area basis, was calculated from three directly measurable morphometric variables (needle length, width, and thickness) measured on freshly collected needles. The total surface area of pine needles was computed assuming the needles to be half cylinders (Berninger and Nikinmaa 1994). The measured needles were divided into subsamples of 10 needles, 10 replications per forest type, and dried at 65°C for 48 h. Subsequently, the sum of the areas of 10 needles was divided by the dry mass of the corresponding sample.

Data analysis

To compare the effects of sampling sites and forest types on the number of needle age-classes, mean survivorship of needles, and length of shoots, a mixed model of two-level nested analysis of variance (ANOVA) was used. Sampling site was treated as a random factor nested within forest types (a fixed factor). Statistical comparisons of the means of N_m and SLA among forest types were conducted by applying a model I one-way ANOVA. To compare needle survivorships of the same needle age-class, we first calculated mean values for each sampling site and then compared them using a Kruskal–Wallis test with forest type as a grouping variable. Post hoc mean comparisons were conducted using the Student–Newman–Keuls (SNK) multiple range procedure. The assumptions of normality and homogeneity of variances were checked using the Kolmogorov–Smirnov D statistic and the Brown–Forsythe test, respectively. All statistical computations were made with the aid of the computer package STATISTICA at the significance level $\alpha = 0.05$ (StatSoft, Inc. 1998).

Results

The mean number of needle age-classes was 4.1–4.2 in the three forest types. The variation among the sampling sites within forest types was highly significant ($p < 0.001$), whereas the effect of forest type was statistically insignificant (Table 2). A maximum of six age-classes of needles (from current-year up to 5-year-old needles) were counted in all forest types; the minimum was two age-classes for pine bog and dry heath forests, and three age-classes for *Vaccinium* forests. Contrary to the effect of forest type on the number of needle age-classes, its effect on mean needle survivorship was significant ($p < 0.01$), while that of sampling sites within forest types was insignificant (Table 2).

The overall mean needle survivorship in *Vaccinium* forests was significantly lower than in the other two forest types (Table 3). The survivorship of the current-year and 1-year-old needles was equal in all forest types, but that of the 2- and 3-year-old needles was significantly lower in *Vaccinium* forests (Fig. 2). The 4-year-old needles had the same survivorship in all forest types, but the needles of the trees growing in *Vaccinium* forests had a greater chance of living

Table 2. Results of the nested one-way analysis of variance for the effect of site and forest type on the number of needle age-classes, mean survivorship of needles, and shoot length increment (mm·year^{-1}).

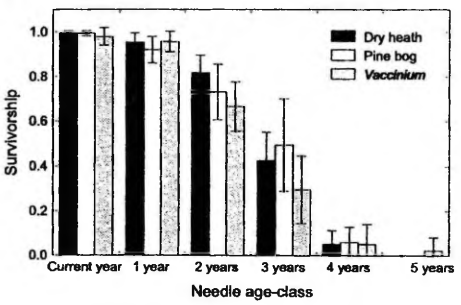
Dependent variable	Source of variation	SS	df	*p*
No. of needle age-classes	Forest type	0.5	2	0.9
	Site (nested in forest type)	50	27	<0.001
	Error	108	270	
Mean survivorship of needles	Forest type	0.227	2	<0.01
	Site (nested in forest type)	0.426	27	0.08
	Error	2.986	270	
Shoot length increment	Forest type	158 173	2	<0.001
	Site (nested in forest type)	89 552	27	<0.001
	Error	307 682	270	

Table 3. Mean needle survivorship, N content of needles (N_m), specific leaf area (SLA), and shoot length increments in the three forest types.

	Vaccinium forests	Dry heath forests	Pine bog forests
Mean survivorship, %	70±1.1*a* (100)	80±1.1*b* (100)	80±1.0*b* (100)
N_m, mg·g^{-1}	11.8±0.85*a* (5)	10.4±0.56*b* (5)	9.8±1.07*b* (5)
SLA, mm^2·mg^{-1}	6.3±0.51*a* (10)	6.0±0.56*a* (10)	5.5±0.44*b* (10)
Shoot length increment, mm·year^{-1}	92.2±9.19*a* (100)	95±46.83*a* (100)	45.1±16.85*b* (100)

Note: Values are means ± SDs, and the number of observations is given in parentheses. Values within rows with different letters are significantly different at $\alpha = 0.05$.

Fig. 2. Survivorship of needles (mean ± SD) of different age-classes in Scots pine in three forest types. The survivorship of the 2- and 3-year-old needles in *Vaccinium* forests was significantly smaller than in the other forest types at the $\alpha = 0.05$ level (Kruskal–Wallis test).



5 years. Thus, the differences in the mean needle survivorship among forest types were due to the smaller survivorship of 2- and 3-year-old needles in *Vaccinium* forests, whereas the decline in the mortality rate of the 4-year-old needles results in the same number of needle age-classes across all forest types.

The nitrogen content of the 1-year-old needles varied significantly among the studied forest types (one-way ANOVA, $F = 6.80$, df = 2, $p = 0.01$). Like the mean needle survivorship, N_m of the needles collected from *Vaccinium* forests differed from that in the pine bog and dry heath forests (Table 3). The specific leaf area and shoot length increment followed the same general trend of variation among the examined forest types. Although the SLA varied significantly among the forest types (one-way ANOVA, $F = 6.66$, df = 2, $p < 0.01$), the results of the SNK test showed that the mean values for dry heath and *Vaccinium* forests were similar and significantly higher than the value for pine bog forests (Table 3).

The forest types explained approximately 28% and sampling sites about 16% of the total variation in the shoot length increment (Table 2). The mean shoot length increment in pine bog forests was significantly smaller than that in *Vaccinium* and dry heath forests. When all the data were pooled, the shoot length increment showed a weak negative but statistically significant ($r = -0.13$, $p < 0.05$) correlation with needle longevity.

Discussion

Our results confirmed that the mean needle survivorship of Scots pine is smaller in a plant community growing on fertile sites (*Vaccinium* forests) as compared with communities on sites with limited resource availability (dry heath and pine bog forests), whereas the number of needle age-classes did not significantly differ between the communities. However, in studies of intraspecific variation of conifers' needle longevity across climatic zones, it has been shown that the maximum life-span of needles is also affected (Ewers and Schmid 1981; Schoettle 1990; Nebel and Matile 1992). We suppose that within the same climatic zone, Scots pine needles may achieve equal maximum age in different habitats, but the mean survivorship of needles tends to be greater at infertile growth sites. The fertilizing experiment performed by Aerts (1989) gave similar results for *Erica tetralix* L.: adding of nutrients caused the leaf survivorship to decrease, whilst the maximum leaf life-span did not change. Consequently, the intraspecific variation of leaf longevity in evergreens is much less within the same climatic zone than it is across climatic zones (months versus years, respectively).

Interspecific comparisons indicated that woody species with smaller leaf longevity have a greater N_m (Reich et al. 1997, 1998, 1999; Eamus et al. 1999; Lusk and Contreras 1999; Kloeppel et al. 2000) and SLA (Gower et al. 1993; Tobin et al. 1999; Warren and Adams 2000) than species with longer leaf longevity under the same growth conditions. This relationship seems to be of a universal nature, since the same trends both in N_m and SLA with respect to leaf longevity have been revealed for perennial herbs as well (Diemer 1998). Intraspecifically, increased needle longevity of Scots pine at higher latitudes or altitudes seems to be phenotypic acclimation to low temperature and nutrient availability, which may be accompanied (but not necessarily) by smaller N_m (Reich et al. 1992, 1996*a*, 1996*b*).

We suppose that greater needle survivorship of Scots pine trees in dry heath and pine bog forests reflects higher resource-use efficiency, and thus can be considered as an acclimation to low nutrient availability. The same foliage is usable for a longer time period, and the plants have to spend fewer resources to form new photosynthetic tissue. Besides, there is the greatest selective pressure in nutrient-poor habitats to maximize the duration of nutrient retention in leaves (Aerts and van der Peijl 1993). Thus, our results are in accordance with those of Aerts et al. (1999), who found that the N_m of the evergreen species from temperate fens and bogs was lower, and nitrogen-use efficiency was higher, than that of the evergreens in fertile environments. Enoki and Kawaguchi (1999) also related intraspecific variation in needle longevity of *Pinus thunbergii* Parl. to the nutrient-use efficiency.

The three forest types considered in the present study were ranked in the same order with respect to the mass-based foliar nitrogen content and SLA of Scots pine: both characteristics were the highest in *Vaccinium* forests, intermediate in dry heath forests, and lowest in pine bog forests. Although both the area and mass of the needles were smaller, the SLA of the trees growing in pine bog forests was still significantly lower compared with the trees in dry heath and *Vaccinium* forests. Thus, the trees growing in waterlogged conditions in pine bog forests invest more biomass per unit of area of photosynthetic tissue, which has been shown to be a typical characteristic of leaves in arid environments (Reich et al. 1999; Bussotti et al. 2000). Wright and Westoby (1999), exploring variations in relative growth rate and morphological traits of woody dicotyledonous species, depending on nutrient availability and rainfall, concluded that the gradients of decreasing nutrients or rainfall actually appear to be variants of a more general "stress" gradient. Their results and those published by Poorter and de Jong (1999), and Castro-Diez et al. (2000) confirmed the same trend that was revealed for Scots pine in the present study: SLA decreased with decreasing resource availability or increasing stress level. In the present case, low nutrient availability and hypoxia are the stress factors combined in waterlogged bog soils. Decrease in SLA in response to low nutrient availability can be considered a universal phenomenon, common for different life forms. Meziane and Shipley (1999) demonstrated the same regularity in their experiments with 22 herbaceous species, and presented a path model confirming direct effect of SLA on both leaf nitrogen levels and net photosynthetic rates (Meziane and Shipley 2001).

Schoettle (1990) has shown that an increase in needle longevity of lodgepole pine (*Pinus contorta* Dougl. ex Loud.) with the altitude of the growth site was accompanied by decreasing shoot length increment. In this study we found that despite longer needle longevity, the annual shoot increments of the trees in dry heath forests did not differ from those of the trees in *Vaccinium* forests. Nevertheless, when all data was pooled, the shoot length increment was negatively correlated ($p < 0.05$) with mean needle longevity.

It has been argued that the distribution of evergreen and deciduous species along soil fertility gradients reflects the advantages of long-living foliage in nutrient-poor environments (Monk 1966; Bazazz 1979; Kloeppel et al. 2000). Although the extent of variation in needle survivorship of Scots pine among forest types was not comparable with interspecific variations in leaf longevity, its ecological significance is the same. For pine trees, a longer period of leaf retention in infertile sites most likely confers higher resource-use efficiency, which is needed to achieve a positive carbon balance at limited resource availability. Alternatively, the community-specific variation in needle survivorship may reflect the longer residence time of nutrients in the foliage of the trees on nutrient-poor soils. However, both of these assumptions need further studies.

The community-specific variation in needle survivorship revealed in this study has an important outcome for forest survey projects. Already in early years of widespread forest decline in Europe, there were claims that natural variation in signs of tree health was unknown (Blank 1985; Innes 1993). In Scots pine, the temporal variation of needle longevity and that across latitudes had been well documented for northern Europe previously (Jalkanen 1998; Jalkanen et al. 1994, 1995, Pensa and Jalkanen 1999; Pensa et al. 2001); the present study widens our understanding of the causes of small-scale spatial variation in needle life-span. This consideration is a crucial point for the establishing of forest survey programmes where needle longevity is used to estimate the condition of coniferous stands.

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Soil type affects nitrogen conservation in foliage of small *Pinus sylvestris* L. trees

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Key words: leaf longevity, nitrogen concentration, nitrogen-resorption efficiency, nitrogen-resorption proficiency, *Pinus sylvestris*, soil type

Abstract

Nutrient conservation in plants and soil fertility may be intricately linked. We studied nitrogen conservation in small Scots pine (*Pinus sylvestris* L.) trees growing in stands on organogenic *Dystric Histosols* and on mineral *Podzols*. Nitrogen-resorption efficiency (NRE) and proficiency (NRP) of senescent needles, and mean residence time of nitrogen (MRT) were studied in relation to needle surface area, needle longevity, and leaf mass per area (LMA). Trees on *Podzols* had higher nitrogen concentration in green needles than the trees on *Dystric Histosols*, but the nitrogen concentration of yellowing needles was similar for trees on both soil types. NRE averaged 65±3.5% (mean±SD) and 56±7.2% for the trees on *Podzols* and *Dystric Histosols*, respectively. Neither NRP (0.44±0.05% and 0.35±0.07%, respectively) nor MRT (8.4±2.3 and 6.1±1.2 years) differed significantly between the stands on the two soil types. Mean needle surface area was significantly smaller in trees on *Dystric Histosols* (76±29 mm^2) than on *Podzols* (131±38 mm^2), whereas needle longevity varied between 2 and 4 years independently of the soil type. Trees invested, on average, the same amount of dry matter per unit of needle area on both soil types. Growth of trees, measured as increment of shoot length, was more restricted on *Dystric Histosols* (55±18 mm yr^{-1}) than on *Podzols* (184±44 mm yr^{-1}). The results of the correlation analysis applied to pooled data were inconsistent with the relations between traits of stress resistance syndrome observed in inter-specific comparisons. The study indicated that Scots pine trees relocated nitrogen from senescent foliage more efficiently on mineral *Podzols* than on organogenic *Dystric Histosols*, but the minimum nitrogen concentration of needles appeared to be similar on both soil types.

Abbreviations: LMA – needle dry mass per unit total area (g m^{-2}); MRT – mean residence time of nitrogen (yr); N_m – mass-based nitrogen concentration of needles (mg g^{-1}); NRE – nitrogen-resorption efficiency (%); NRP – nitrogen-resorption proficiency (%)

Introduction

Many plant ecologists have considered the resorption of nutrients from senescent parts of plants a primary nutrient-conservation mechanism, which enables the reuse of nutrients once accumulated in plant tissues (Aerts, 1996; Chabot and Hincks, 1982; Chapin, 1980; Eckstein et al., 1999), Besides resorption, nutrients can be conserved by extending the life span of plant organs. Among 14 subarctic species studied by Eckstein and Karlsson (1997), the woody evergreens demonstrated the longest MRT and the lowest nutrient productivity. These two properties are often accompanied by a suite of traits called 'stress resistance syndrome', which can be treated as an adaptation to stressful conditions (Chapin et al., 1993). Studies comparing leaf life spans, nutrient use, and photosynthetic rates across various biomes or ecosystems have

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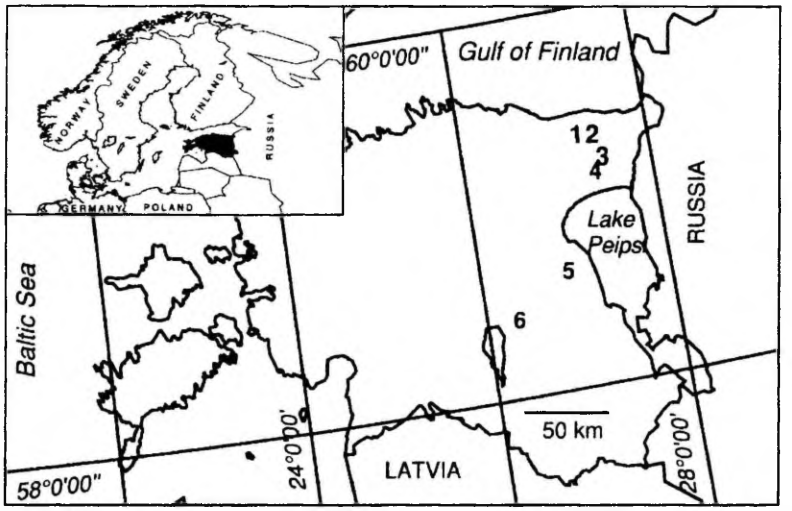

Figure 1. Location of the sampling sites has been marked with numbers. The inserted map shows the location of Estonia (the black area).

shown that these characteristics are strongly related to each other and the relationships are valid within a wide range of habitats (Reich et al., 1992, 1999).

However, it is not evident that adjusting leaf longevity to stress conditions within a species should be associated with a decrease in productivity and growth rate (Eckstein and Karlsson, 2001), although within-species comparisons across climatic zones have shown such an inverse relationships (Jalkanen et al., 1995; Kikuzawa, 1991; Lamhamedi and Bernier, 1994; Weidman, 1939). Nonetheless, besides variation across climatic zones (along altitudinal or longitudinal gradients), leaf longevity also varies within a climatic zone if individuals of the same species are subjected to different growth conditions (Aerts, 1989). Whether changes in nutrient conservation accompany this small-scale within-species variation in leaf life span is rather unclear. In about 60% of the intra-specific experiments, nutrient-resorption efficiency (defined as a percentage of nutrients relocated from senescent tissues) did not respond to increased nutrient availability (Aerts, 1996). Nordell and Karlsson (1995) presented data that habitats affected nitrogen resorption in mountain birch, but it remained unclear whether this variation was related to habitat fertility. In radiata pine, the resorption efficiency of nitrogen was not connected with soil nutrient availability (Nambiar and Fife, 1987). An experiment carried out on Alaskan birch also showed that the nutrient status of the trees did not affect the nitrogen-resorption process (Chapin and Moilanen, 1991). However, there is a number of published studies which report that nutrient resorption within a species decrease in response to enhanced soil fertility; about 30–35% of the studies analysed by Aerts (1996) gave such results (see also Kost and Boerner, 1985). Thus, within a species it is still unknown to what degree nutrient conservation (e.g. MRT) depends on variation either in nutrient-resorption efficiency or in longevity of different plant parts (Eckstein et al., 1999). Among species longevity explains more variation in MRT than nutrient resorption, while, within species, resorption appears more relevant than biomass longevity (Eckstein et al., 1999).

In the present study, we compare nitrogen conservation in terms of resorption efficiency (percentage of a nutrient withdrawn from leaves prior to abscission), resorption proficiency (concentration to which a nutrient is reduced in senescent leaves), and mean residence time of nitrogen and needle morphology of Scots pine (*Pinus sylvestris*) trees growing on well-drained mineral soils classified as *Podzols* and on waterlogged organogenic soils classified as *Dystric Histosols*. The definitions of the characteristics used are given by Berendse and Aerts (1987), Eckstein et

Table 1. Basic characteristics of the sampling sites. N_m, overall average nitrogen concentration of needles

Site no.	Co-ordinates	Soil type	Depth of water table, m	Soil total N, mg g^{-1} (mean ± SD)	N_m, mg g^{-1}	NRE,%	MRT, yr	Shoot growth rate, mm yr^{-1}
1	59° 10′ N 27° 17′ E	Dystric Histosols	0.20	0.7±1.7	7.3±2.5	56±7.1	7.1±1.0	54±28
2	59° 05′ N 27° 28′ E	Dystric Histosols	0.15	0.6±1.1	5.8±1.9	56±7.4	5.9±0.9	55±12
3	58° 26′ N 27° 16′ E	Dystric Histosols	0.20	0.6±0.8	5.8±2.2	58±8.4	5.3±1.2	57±13
4	59° 10′ N 27° 27′ E	Podzols	>2	1.3±0.7	9.8±3.9	63±3.1	7.4±1.5	183±34
5	59° 06′ N 27° 28′ E	Podzols	>2	0.6±0.1	8.5±3.5	67±2.8	10.7±1.9	150±39
6	58° 40′ N 26° 52′E	Podzols	>2	1.1±0.5	9.6±3.9	66±4.0	7.0±1.6	219±29

al. (1999) and Killingbeck and Whitford (2001). Scots pine is a coniferous species that forms stands under a wide range of soil conditions, but, despite the great economic importance of the species, few studies (Escudero et al., 1992; Helmisaari, 1992; Näsholm, 1994) have examined the nutrient economy of Scots pine trees in different habitats. The specific questions we addressed are: (1) do resorption efficiency, proficiency, and mean residence time of nitrogen in foliage of pine trees growing on *Dystric Histosols* differ from those in trees on *Podzols*? (2) do differences in MRT result from variation in nitrogen-resorption efficiency or leaf life-span? (3) are resorption and conservation of foliar nitrogen related to other traits of pine needles?

Materials and methods

Study site

The study was conducted in Estonia, where climatic conditions favour the prevalence of boreal mixed forests. Mean annual temperature in Estonia ranges from 6.0 °C in the western part to 4.0 °C in the eastern part. Annual precipitation fluctuates between 500 and 700 mm, being greater in southeastern Estonia due to its relatively higher relief (maximum elevation in Estonia is 318 m a.s.l.).

During the summer of 2001, we estimated the nitrogen-resorption efficiency and proficiency, needle longevity, needle area and leaf specific mass (LMA) in 6 Scots pine (*Pinus sylvestris* L.) stands growing on two different soil types classified by FAO-UNESCO as *Dystric Histosols* and *Podzols* (3 study sites per each soil type). The studied stands were selected within narrow latitudinal and altitudinal zones to eliminate the possibility that differences in needle longevity and related traits might be caused by climatic factors. The stands on *Podzols* belonged to *Vaccinium* forest type and those on *Dystrict Histosols* to pine bog forest type. The forest types have been differentiated by a complex of indices related directly or indirectly to soil fertility and water availability (Masing, 1996). The corresponding forest types according to Cajander's (1926) classification, widely usable in the Nordic countries, are *Vaccinium* and pine peat moor types, respectively. Basic characteristics of the study sites are given in Table 1.

Sampling

A total of 30 Scots pine trees were sampled at 6 sites (5 trees per site) between latitudes 58° 00′ N and 59° 30′ N (Figure 1), the altitude of the sites ranged from 50 to 100 m a.s.l. The height of the sample trees varied between 1.5 and 3 m on both soil types. The canopies at all sites were open and the crowns of the sample trees were mostly exposed to direct sunlight. At all sites, the number of needle age classes was counted on two branches in the mid-part of the northern side of the sample trees' crowns. The number of needle age classes was used for estimating maximum needle longevity, as described by Ewers and Schmid (1985).

For analysing mass-based nitrogen concentration (N_m, mg g^{-1}) and nitrogen conservation in foliage, we collected needles from two branches of each sample tree three times during the growth period. We sampled green needles from the oldest needle age classes (referred to as **Set 1**) in June, just after the sprouting of new needles. The current year needles were collected at the end of July (**Set 2**), and the yellowing needles from the oldest age classes just before abscission in September (**Set 3**). The N_m was assessed according to Kjeldhal's method at the Plant Biochemistry Labor-

atory of the Estonian Agricultural University (Tartu, Estonia). For estimating total nitrogen concentration of soil, three soil samples were taken at each sampling site from a depth of 0–0.15 m. To characterise general growth potential of trees on both soil types, we measured length of one-year-old shoots on two branches of each sample tree.

Nitrogen conservation and needle morphology

Nitrogen conservation was characterised by nitrogen-resorption efficiency, proficiency, and mean residence time (Killingbeck and Whitford, 2001). The nitrogen-resorption efficiency (NRE) is the percentage of the element removed from senescent leaves, and it was calculated as follows:

$$NRE = \frac{(N_{m1} - N_{m3}) \cdot 100}{N_{m1}}, \quad (1)$$

where N_{m1} and N_{m3} are leaf nitrogen concentrations (mg g^{-1}) of **Sets 1** and **3**, respectively. The nitrogen-resorption proficiency (NRP) is the level to which a nutrient has been reduced in the senescent leaves, and it was expressed as the concentration (%) of nitrogen in **Set 3**. The mean residence time of nitrogen (MRT) was calculated according to Eckstein et al. (1999):

$$MRT = \frac{N_{m2} \cdot A_{max}}{N_{m3}}, \quad (2)$$

where N_{m2} is leaf nitrogen concentrations (mg g^{-1}) of **Sets 2**, A_{max} is maximum needle longevity (years).

Five to 10 needles per tree were randomly taken from each foliage sample to measure needle width and thickness with precise caliper (±0.01 mm) in the top, central, and basal parts of the needles. The average of these estimates was employed to compute the needle cross-sectional circumference, assuming the needle cross section to be elliptic (Niinemets et al., 2001). Needle length was measured with a ruler, and the total surface area was calculated as the product of needle circumference and length. The mass of the measured needles was determined after drying at 65 °C for 48 h, and needle dry mass per unit total area (LMA, g m^{-2}) was calculated.

Data analysis

To compare the effects of sampling sites and soil types on needle longevity, N_m, NRE, NRP, MRT, needle surface area, and LMA, a mixed model of two-level nested analysis of variance was used. Sampling site was treated as a random factor nested within soil types (a fixed factor). The assumptions of normality and homogeneity of variances were checked using the Kolmogorov-Smirnov D-statistic and the Brown-Forsythe test, respectively. All statistical computations were made with the aid of the computer package Statistica'98 (StatSoft Inc., Tulsa, USA) at the significance level α=0.05 (Statistica, 1998).

Table 2. Results of the nested two-level analysis of variance for effects of site and soil type on needle surface area, LMA, and maximum needle longevity. df, degrees of freedom; *p*, statistical significance

Dependent variable	Source of variation	df	*p*
Surface area	Soil type	1	0.003
	Site (nested in soil type)	4	0.6
LMA	Soil type	1	0.7
	Site (nested in soil type)	4	0.6
Maximum needle longevity	Soil type	1	0.6
	Site (nested in soil type)	4	<.001

Results

Soil nitrogen concentration varied between 0.6 and 1.3 mg g^{-1}. However, this variation can not be related to soil type because one of the three sites on *Podzols* had the same soil nitrogen concentration as two sites on *Dystrict Histosols* (Table 1). Growth potential of trees was significantly higher on *Podzols* (average shoot growth rate was 184±44 mm yr^{-1}) than on *Dystrict Histosols* (55±18 mm yr^{-2}; $p<0.01$, two-level nested ANOVA).

Surface area of the needles varied significantly between soil types but not among sampling sites (Table 2). Pines growing on organogenic soil (*Dystrict Histosols*) averaged 1.6 times smaller surface area than pines on mineral soil (*Podzols*) (76±29 and 131±38 mm^2, respectively). Needle mass per area (LMA) did not differ significantly between soil types nor among sampling sites, ranging from 50 to 200 g m^{-2} (Table 2). Maximum needle longevity varied between 2 and 4 years, independently of soil type, but the variation resulting from differences in sampling sites and individual trees was highly significant (Table 2).

The nitrogen concentration of the needles depended on needle age (sets), soil type, and sampling

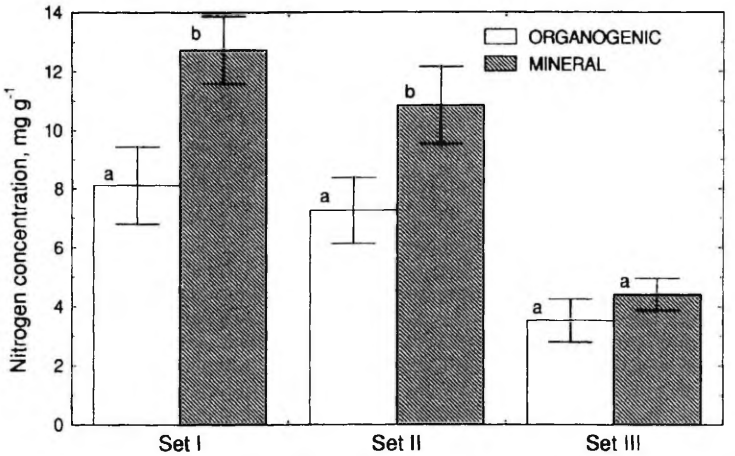

Figure 2. Nitrogen concentration of needles (mean±SD) of different ages in Scots pine on two soil types. Set I – green needles of the oldest age class (June), Set II – current-year needles (July), Set III – yellowing needles of the oldest age class (September). Bars marked with different letters were significantly different at $\alpha = 0.05$ level.

Table 3. Results of the nested three-level analysis of variance for effect of needle set, soil type and sampling site (nested in soil type) on N_m. df, degrees of freedom; *p*, statistical significance

Source of variation	df	*p*
Needle set	2	<0.001
Soil type	1	0.008
Site (nested in soil type)	4	<0.001
Needle set × soil type	2	<0.001
Needle set × site	8	0.2

Table 4. Results of the nested two-level analysis of variance for effect of sampling site and soil type on nitrogen-resorption efficiency, resorption proficiency and MRT. df, degrees of freedom; *p*, statistical significance

Dependent variable	Source of variation	df	*p*
N-resorption efficiency	Soil type	1	0.002
	Site (nested in soil type)	4	0.8
N-resorption proficiency	Soil type	1	0.06
	Site (nested in soil type)	4	0.06
MRT	Soil type	1	0.2
	Site (nested in soil type)	4	0.02

site (Table 3). The interaction of needle sets and soil type also significantly affected N_m (Figure 2). Within soil types, the N_{m3}(4.4±0.5 mg g^{-1} on the mineral and 3.5±0.7 mg g^{-1} on the organogenic soil) was significantly lower than $N_{m1}$and N_{m2}. N_{m3} did not significantly differ between the two soil types. On organogenic soil, the N_{m1} (8.1±1.3 mg g^{-1}) did not differ from that of N_{m2} (7.2±1.1 mg g^{-1}), while both were significantly smaller than the N_m of green needles on the mineral soil. The highest level of N_m was recorded in **Set I** for the trees growing on the mineral soil (12.7±1.1 mg g^{-1}), followed by the N_{m2} (10.8±1.3 mg g^{-1}). The overall average N_m for trees on organogenic soil was 6.3±2.3 mg g^{-1}, and for those on mineral soil, 9.3±3.7 mg g^{-1}.

Trees growing on mineral soil exhibited nearly 10% higher nitrogen-resorption efficiency as compared to trees on organogenic soil (65±3.5 and 56±7.2%, respectively). Nitrogen-resorption proficiency did not significantly differ between soil types (0.35±0.07% for the organogenic and 0.44±0.05% for the mineral soil). As with NRE, the effect of sampling site on NRP was not statistically significant (Table 4). Mean residence time of nitrogen in pine needles was 8.4±2.3 years for the stands on the

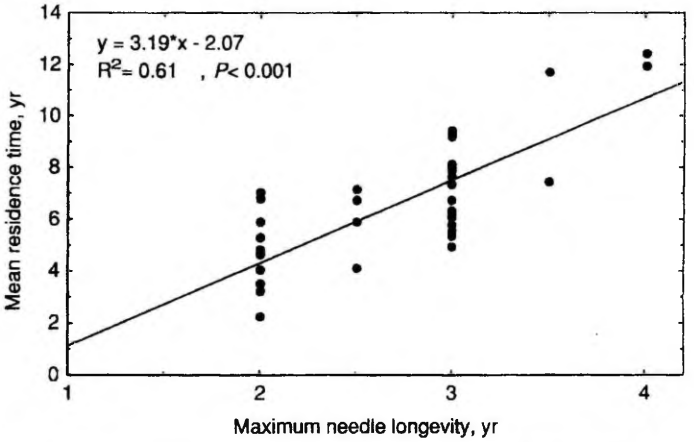

Figure 3. Mean residence time of nitrogen in pine foliage as a function of maximum needle longevity across all the six sampling sites.

mineral soil and 6.1±1.2 years for those on the organogenic soil. Nevertheless, the difference between MRT for the trees on the different soil types was not significant (Table 4). Site-specific data on overall average nitrogen concentration of needles, NRE, and MRT are given in Table 1.

Across all the six sampling sites, the N_{m2} was positively correlated with N_{m1} and the N_{m3} (Table 5). The greater the surface area of the needles, the higher was the N_m of the green leaves. Mean residence time was positively related to maximum needle longevity (Figure 3) and needle area. Maximum needle longevity was negatively correlated with LMA.

Discussion

Scots pine can cope with severe shortages of nutrients and water or with an excess of soil moisture. According to Grime (1977), it belongs to a stress-tolerant strategy type, reassessed by Grubb (1998) as a switching strategy type. As Scots pine occupies a wide range of habitats, the trees vary in phenotype. For example, pines growing on infertile sites are rather small and exhibit low growth rates, while in fertile sites they are large and fast growing. Needle traits also differ significantly depending on soil conditions. Our study revealed that needle surface area was 1.6 times smaller for trees growing on the organogenic soil (*Dystric Histosols*) compared to trees on mineral soil (*Podzols*). The same tendency was observed in our previous study (Pensa and Sellin, 2002), and it is probably related to both low nutrient availability and hypoxia, resulting from waterlogged conditions, in *Dystrict Histosols*. The stands growing on the two sites of differing soil conditions did not differ significantly in LMA. This contradicted our previous data indicating that specific leaf area (i.e. inverse of LMA) of pine needles declined with decreasing site fertility (Pensa and Sellin, 2002). The disagreement probably results from a high variability of LMA between the sites and trees, while only three stands per soil type (versus 10 sites per forest type in the previous study) were sampled in the present study.

Foliage nitrogen concentration expressed per mass of green needles was clearly greater in trees on mineral soil being the highest in mature green needles (N_{m1}). On organogenic soil, the current-year needles had the same level of nitrogen concentration as old green needles, but on mineral soil the nitrogen concentration was greater in old green needles than in current-year needles. Studies concerning the nutrient content in foliage of different ages have confirmed that current-year needles have the highest nitrogen level (Florence and Chuong, 1974; Hom and Oedul, 1983; Nambiar and Fife, 1987). Therefore it is most plausible that current year needles (**Set 2**) had not achieved the maximum value of N_m by the end of July when they were sampled.

Table 5. Correlation matrix of the foliar nitrogen conservation characteristics and needle traits across all the sampled trees at the six sampling sites. Significant correlations are marked with asterisk

	N_{m1}	N_{m2}	N_{m3}	NRE	MRT	Max. needle longevity	Area
N_{m2}	0.89*	–					
N_{m3}	0.58	0.61*	–				
NRE	0.47	0.31	–0.44	–			
MRT	0.35	0.39	–0.22	0.57	–		
Max. needle longevity	0.10	0.14	0.05	–0.02	0.77*	–	
Area	0.76*	0.71*	0.54	0.21	0.63*	0.66*	–
LMA	–0.05	0.12	–0.35	0.37	–0.28	–0.61*	–0.47

Trees growing on mineral soil were more efficient at resorbing nitrogen from senescent needles than trees growing on the organogenic soil. Mean nitrogen-resorption efficiency in foliage for the pines growing on the mineral and organogenic soils (65% and 56%, respectively) was consistent with most of the data published by other authors. In the study carried out by Näsholm (1994) on Scots pine, the NRE varied between 45 and 65%, and in that by Helmisaari (1992) it ranged from 40 to 70%. In studies carried out on other pine species, NRE varied between 33 and 62% (Dalla-Tea and Jokela, 1994; Escudero et al., 1992; Nambiar and Fife, 1987). Across a wide range of evergreen shrubs and trees the NRE in foliage averaged 47% (Aerts, 1996).

Nitrogen-resorption proficiency of Scots pines was similar for both soil types: on average, 0.44% for *Podzols* and 0.35% for *Dystric Histosols*. These values were remarkably smaller than mean nitrogen concentrations in the senescent leaves of many deciduous and evergreen woody species published by Chapin and Kedrowski (1983; 0.82%) and by Killingbeck (1996; 0.87%). Using the NRE estimations for Scots pine made by Escudero et al. (1992) and Näsholm (1994), Killingbeck (1996) found the respective values of NRP to be 0.52% and 0.50%, respectively. Our findings indicate that Scots pine is able to reduce N to even lower concentrations in its senescent needles, and apparently complete resorption of nitrogen (or ultimate resorption proficiency, defined as a maximum level to which nitrogen can be reduced in senescent leaves of any species; Killingbeck, 1996) took place on both soil types. Killingbeck (1996) stated that NRP appeared to be especially useful term in addressing questions related to the selection pressures that have influenced nutrient resorption process in plants. Nutrient-resorption proficiency values are more definite and objective measures of the degree to which plants can minimise nutrient loss. Nitrogen-resorption efficiency values suit better for describing a relative degree to which plants can conserve nutrients invested in foliage. It encompasses both nutrient demand and nutrient withdrawal.

Scots pine, as a light-demanding species, is distributed on a wide variety of soil types, being restricted on more fertile sites by fast-growing tree species that are better competitors for light. However, its ability to manage with a low level of nutrients or oxygen gives Scots pine an advantage in adverse soil conditions. Compared with many other species (Chapin and Kedrowski, 1983; Killingbeck, 1996), Scots pine is more proficient at resorbing nitrogen from senescent leaves. In more fertile soil types, pines achieve a higher level of nitrogen in foliage than in poor sites, but the level to which the nitrogen concentration of the senescent needles declines seems to be similar in different habitats. This explains why there was a significant variation in NRE between soil types, but not in NRP. The same is valid in case of inter-specific variation: for example, the NRE of understory shrub species growing in an oak-dominated forest differed among species, but NRP did not (Killingbeck and Costigan, 1988). However, this inverse juxtaposition of NRE and NRP did not hold in shrub species inhabiting the margins of a desert arroyo (Killingbeck and Whitford, 2001).

The mean residence time of nitrogen did not statistically differ between pine stands on the different soil types, although the resorption efficiency was higher

on mineral soil. However, variation in NRE did not cause equidirectional variation in MRT, because the maximum life span of needles was distributed uniformly between the soil types. Eckstein et al. (1999) have argued that resorption efficiency is more important in explaining variation in MRT at the intra-specific level than at the inter-specific level. The increase in resorption efficiency compensates for a twofold increase in maximum needle longevity (Eckstein et al., 1999), but in this study the 10% difference in resorption efficiencies between the soil types was not big enough to differentiate MRT of nitrogen in the foliage of the trees growing in the mineral and organogenic soils. The analysis of the pooled data across all study sites indicated that MRT of nitrogen in pine foliage was primarily determined by needle longevity (Figure 3). Escudero et al. (1992), studying the simulated effects of resorption efficiency on MRT at different leaf longevities, proposed that only NRE values above 80% would cause MRT of nitrogen in short-lived leaves to increase more than the lengthening of longevity of the foliage with smaller resorption efficiency.

When data on both soil types were pooled, trees with higher nitrogen concentration in current year needles were less proficient in resorbing nitrogen than the trees with low nitrogen concentration (positive correlation between N_{m2} and N_{m3} in Table 5). Thus, trees with higher N_{m2} lost more nitrogen with abscission of old needles. The NRE was not related to N_m of green needles, which agrees with the results of Chapin and Moilanen (1991) and Escudero et al. (1992), but not with those by Nordell and Karlsson (1995). However, a wider range of N_m values should be used to draw reliable conclusions about the nutritional control on NRE of Scots pine needles. Neither the correlation between NRE and MRT or between MRT and N_m was statistically significant. The positive relationship between needle longevity and needle area as well as the negative correlation with LMA are somewhat surprising results in the light of the theory of stress resistance syndrome (SRS), which states that in infertile habitats the plants have scleromorphic leaves, slow growth rate, low photosynthetic rate and low leaf turnover rate (Chapin et al., 1993). The pine trees growing under stress conditions (low growth rate on waterlogged organogenic soil) had needles with smaller surface area, which, according to SRS, should be accompanied by longer leaf life-span and higher LMA. However, the correlation analysis showed that the greater the needle surface area (which was more likely in the case of the pines on the mineral soil) the longer the needles' life span tended to be. As the links between leaf structure, leaf longevity, and stress resistance have been found mostly in inter-specific comparisons (Chapin et al., 1993; Reich et al., 1992), the intra-specific relationships may be of a different nature. Thus, Eckstein and Karlsson (2001) could not find a trade-off between MRT and nutrient productivity within species, although that was observed between species. The variation of the leaf traits connected to SRS within a single species is probably too small to exhibit the apparent relations between leaf properties, while at the inter-specific level, the expected relationships are likely to be more pronounced, since wider ranges of values will be compared. Positive correlation between needle surface area and their maximum longevity observed in this study is most likely caused by the fact that bigger needles are more characteristic to healthy, well-growing trees which are able to keep their foliage for a longer period. However, more studies need to be carried out on different species that are distributed across a wide range of habitats in order to clarify the intra-specific relationships between leaf life span, leaf morphology and nutrient use strategy.

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VI

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Variation in needle traces diameter in respect
of needle morphology in five conifer species.
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VARIATION IN NEEDLE-TRACE DIAMETER IN RESPECT OF NEEDLE MORPHOLOGY IN FIVE CONIFER SPECIES

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Abstract Needle traces have been used for revealing historical changes in needle longevity of *Pinus sylvestris* L. and *Picea abies* (L.) H. Karst. In this paper we compared the needle-trace diameter of five conifer species (*Abies sibirica* Ledeb., *Picea abies, Pinus sylvestris, Pseudotsuga menziesii* (Mirb.) Franco and *Taxus baccata* L.) in respect of needle size. In case of *P. sylvestris*, we studied how needle-trace diameter varied among different growing sites and among different shoots within a site, and related that variation to needle morphology and structure. *Pinus sylvestris* had the greatest diameter of needle traces (233±30 μm) followed by *T. baccata* (141±19 μm) and *P. menziesii* (121±30 μm). The smallest needle-trace diameter was in *A. sibirica* (85±21 μm) and *P. abies* (80±18 μm). Among species, the needle-trace diameter was strongly correlated with needle length ($r=0.93$, $p<0.05$). Within *P. sylvestris*, the needle-trace diameter was significantly affected by location ($p<0.001$) and varied significantly among shoots within a location ($p<0.001$). The shoots of *P. sylvestris* which had greater average diameter of needle traces, tended to have longer ($r=0.36$, $p<0.05$), wider ($r=0.55$, $p<0.05$) and heavier ($r=0.64$, $p<0.05$) needles with greater stomatal density ($r=0.55$, $p<0.05$). Although our results do not allow using needle-trace diameter in predicting properties of needle structure at the present stage, they nevertheless indicate general trends and point to a need for further studies in this topic.

Key words: Coniferous trees, Needle trace, Needle morphology, Pinus sylvestris

INTRODUCTION

Apart from their use in connection with tree rings, there are only a few other examples of research in which the characteristics or properties of wood structure have been used to illustrate environmental changes. One such example is the Needle Trace Method (NTM) (Kurkela and Jalkanen 1990). It focuses on the needle traces of conifers, embedded in the innermost tree rings, from the pith outwards. Needle traces are defined as part of the vascular system that connects needles with the main vascular system of the stem (Eames and MacDaniels 1935; Elliott 1937). By determining the length of the needle traces

(in how many tree rings a particular needle trace occurs), the age of needles can then be estimated. The NTM is used to produce historical reconstruction of needle retention in connection with climate conditions (Jalkanen et al. 1994a; 1995), air pollution (Jalkanen 1996), pathogens (Jalkanen et al. 1994b; Jalkanen and Aalto 2001) and sylvicultural practices (Jalkanen and Levanic 2001). Besides *Pinus sylvestris* L., NTM has also been applied to *Picea abies* (L.) H. Karst. (Sander and Eckstein 1994; 2001) and other pine species (Jalkanen et al. 2000). Additional to needle retention, it produces data on the history of the needles' general dynamics (needle production, overall number of needles, shedding), which has been used to determine the influence of trees ageing on needle dynamics (Pensa et al. 2001).

Development of leaves is closely related to the development of corresponding leaf traces, as shown by several studies (Larson 1975; 1984; Ewers 1982). The development of leaves vascular pattern is related to leaf elongation and to the formation of epidermal cells (Nelson and Dengler 1997). The leaf vascular system, however, emerges directly from the stem vascular system. It is unclear whether this developmental integration allows conclusions to be drawn about leaf morphological characteristics based on stem vascular system as NTM reveals the history of needle retention. Changes in leaf morphology during the life cycle of trees have become more important due to changes in global climate and atmospheric composition. In this paper, we address the following question: (1) whether needle-trace diameter varies among species in respect to needle morphology (length, width, and thickness), (2) whether the variation in needle-trace diameter within *P. sylvestris* is significant among different sampling sites, among different shoots within a site, and among different needle traces among a shoot, and (3) to what degree the needle-trace diameter correlates with needle morphology and structure (stomatal density) in *P. sylvestris*.

MATERIAL AND METHODS

Sampling and measuring

For comparing needle traces among species, we sampled material from the dendrological park in Toila, Estonia (Fig. 1). In total, five species were sampled (*P. sylvestris*, *P. abies*, *Abies sibirica* Ledeb., *Pseudotsuga menziesii* (Mirb.) Franco and *Taxus baccata* L.). Except for *T. baccata*, which was represented by two samples, five samples represented all other species. Two 3–4-year-old shoots were collected from each tree (five shoots from both samples of *T. baccata*). Thus, ten shoots represented each species.

Variation in needle-trace diameter within *P. sylvestris* was measured on samples taken from six natural sites, four of them in Finland and two in Estonia (Fig. 1). One or two 2–3-year-old shoots were sampled from each tree, five trees per site. Altogether, 600 pine shoots were sampled.

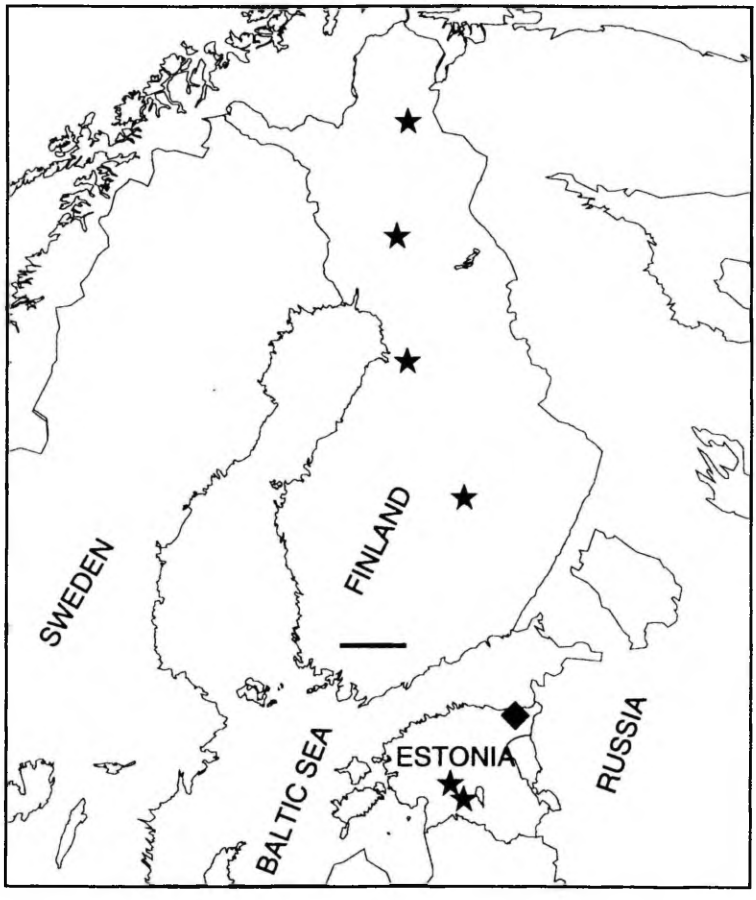

Fig. 1 Location of the sampling sites: (◆) inter-specific comparison; (★) sites of *Pinus sylvestris*. Scale bar – 100 km.

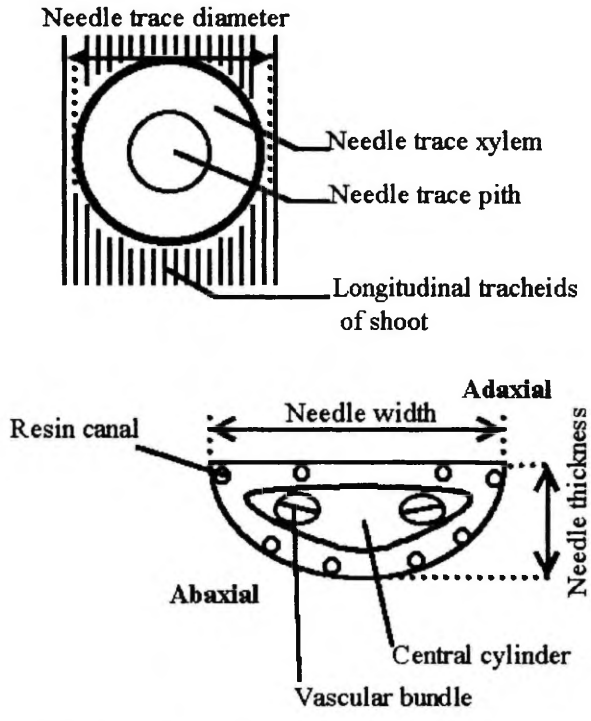

Fig. 2 Cross-sectional schematic diagram of a *Pinus sylvestris* needle trace and needle illustrating the different characteristics measured.

In all shoots we measured the diameter of five needle traces in the wood of the last tree ring for inter-specific comparison, and in the second tree ring in case of comparison within *P. sylvestris*. The diameter was measured in cross-direction with longitudinal axis of long shoots. Thereafter the needles were measured for needle width and thickness in the middle of the needles (Fig. 2). In case of *P. sylvestris*, some needles were additionally studied for stomatal density by counting adaxial stomata in 1 mm section in the middle of the needles . Measurements were made with a stereomicroscope at 50× magnification by placing a 7 mm scale in the eyepiece of the microscope. The length of corresponding needles was measured with a ruler to the nearest mm.

Data analysis

Three-way nested ANOVA was used for estimating the variation in needle-trace diameter among species, among shoots within a species, and among needles within a shoot. All factors (species, shoot and needles) were treated as random.

For comparing needle traces within *P. sylvestris*, 3-way nested ANOVA was used for estimating variation among sites, among shoots within a site and among needles within a shoot. All three factors were treated as random. *Post hoc* mean comparisons were conducted using the Student-Newman-Keuls (SNK) multiple range procedure. The assumptions of normality and homogeneity of variances were checked using the Kolmogorov-Smirnov D-statistic and the Brown-Forsythe test respectively. Pearson's correlation coefficient was used to estimate the strength of relationships between needle-trace diameter and characteristics of needles' morphology. All statistical computations were made with the aid of the computer package Statistica'98 (StatSoft Inc., Tulsa, USA) at the significance level α=0.05 (Statistica 1998).

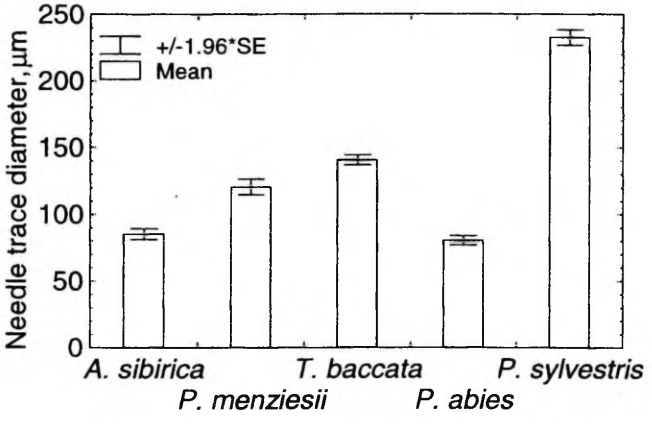

Fig. 3 Variation of needle-trace diameter among species. Whiskers indicate 95% confidence limits for mean values (±1.96*standard error).

Table 1 Results of the nested three-way analysis of variance for the effect of species, sampling shoot, and needles on the needle-trace diameter. SS, sums of squares; df, degrees of freedom; *p*, statistical significance.

Source of variation	SS	df	*p*
Species	1517741	4	<0.001
Shoot (nested in species)	121848	45	<0.001
Needle	76998	200	0.3
Error	88934	250	

RESULTS

Variation among species

The diameter of needle traces varied significantly among species and among shoots of the same species, but not among needles of the same shoot (Table 1). Among the species studied, *P. sylvestris* had the greatest diameter of needle traces (233±30 μm) followed by *T. baccata* (141±19 μm) and *P. menziesii* (121±30 μm). *Picea abies* and *A. sibirica* had the smallest needle traces (80±18 μm and 85±21 μm, respectively); there were no significant differences between these species (Fig. 3). Across species, the diameter of needle traces was strongly correlated with needle length ($r=0.93$, $p<0.05$, Fig. 4a). Correlation between the diameter of needle traces and other characteristics of needle morphology (width, thickness) was not significant.

Variation within *P. sylvestris*

The diameter of needle traces varied significantly among sampling sites, among shoots within the sites and among needles within shoot (Table 2). Except needle thickness, all other measured needle proxies gave a significant positive correlation with the diameter of needle traces. The correlation was strongest between the mean diameter of needle traces and the mean weight of needles (Fig. 4b). The correlation between the diameter of needle traces and the average length of needle pair ($r=0.35$, $p<0.05$) was weakest, but still significant. The strength of correlation between needle-trace diameter and the average width of needles and average density of stomata was 0.55 (Fig. 4c,d).

Discussion

Although our results cannot conclusively be used to predict characteristics of needle morphology from needle-trace diameter, they do point to some general trends that should justify greater attention in further studies. The development of leaves' and shoots' vascular system has been rather well studied, especially in case of Angiosperms (Larson 1975; Larson 1984; Nelson and Dengler 1997; Xia and Steeves 2000; Pizzolato and Sundberg 2002 as some examples). Fewer studies have concerned Gymnosperms (Elliott 1937; Ewers 1982; Boddi et al. 2002). The relationship between needle morphology and needle traces, however, seems not to have been addressed.

The inter-specific differences in needle-trace diameter among studied species are related to needle length. However, needle traces of *P. sylvestris*, a species that has the longest needles among the studied species, have some anatomical

differences compared with other species. In pine genera, the needles are attached to long shoots via short shoots whereas the needles of *Picea* or *Pseudotsuga* have no short shoots. Thus the needle traces of pine species are actually shoot traces and include pith that is surrounded by xylem (Kurkela and Jalkanen 1990; Jalkanen 1995). Therefore the needle-trace diameter of *P. sylvestris* might not be greater because of long needles but because of its anatomical properties. Nevertheless, when *P. sylvestris* was excluded, then the four other species still showed a strong correlation (r=0.84) between needle size (needle width x needle length) and needle-trace diameter. (Results are not presented.) Thus, inter-specific variation in needle-trace diameter is related to needle morphology, and therefore it is easier to use species with bigger needles for NTM studies.

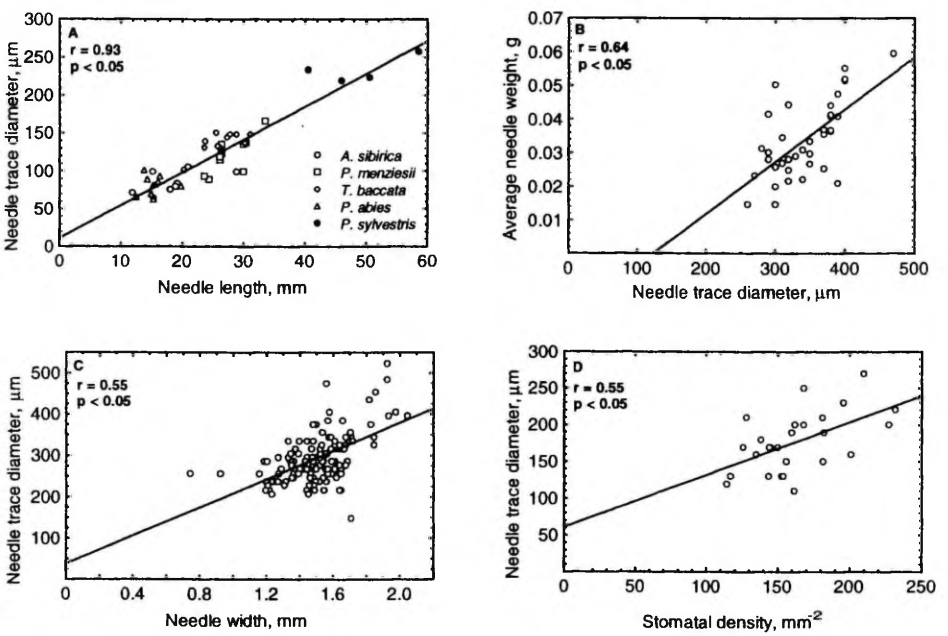

Fig. 4 Correlation of needle-trace diameter with different characteristics measured. a) Across species with needle length; b) within *Pinus sylvestris* with dry weight of needles; c) within *P. sylvestris* with needle width; d) within *P. sylvestris* with stomatal density.

Table 2 Results of the nested three-way analysis of variance for the effect of sampling site, sampling shoot and needles on the diameter of needle traces. SS, sums of squares; df, degrees of freedom; *p*, statistical significance.

Source of variation	SS	df	*p*
Site	819887	5	<0.001
Shoot (nested in site)	75175	18	<0.001
Needle	143855	96	<0.001
Error	85619	120	

In *P. sylvestris*, the needle-trace diameter varied according to the growing site, to individual trees and to individual shoots. This variation was related to needle size, although not so strongly as in the case of inter-specific comparison. In several studies it has been shown that elevated atmospheric CO_2 affects the morphology and structure of plant leaves by decreasing stomatal density (Pearson et al. 1995; Woodward and Kelly 1995; Lin et al. 2001) and increasing needle thickness and the relative area of phloem (Lin et al. 2001). Our results conclude that needle traces restore some information about needle structure, and that this information can be obtained by using NTM. Although the strength of the relationships was not enough to draw direct predictions, further study of this topic is justified in the light of findings presented in this paper. For example, we found a positive correlation between stomatal density and needle-trace diameter. However, not the diameter of needle trace but the area of xylem in the vascular bundle might give stronger relations with stomatal density and can be used for analysing historical changes in it by revealing needle traces in wood. As such studies are not yet carried out, we can only state that the bigger the needle-trace diameter a shoot of *P. sylvestris* has, the greater the stomatal density, width, length and dry weight of the needles attached to the shoot tend to be.

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